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MAJOR ECONOMIC FORCES AFFECTING AGRICULTURE

With Particular Reference to California

S. V. CIRIACY-WANTUP

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Giannini Foundation of Agricultural Economics

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CONTENTS

	Page		Page
1. Introduction	1	7. Factors affecting changes of nonagri-	
2. Trend and fluctuations of cash farm		cultural income	43
income since 1910	2	Summary	43
Summary	2	Processes of income formation	43
Changes of cash farm income	2	Meaning of investment	44
Trend of total cash farm income...	4	Equilibrium between investment and	
Fluctuations of total cash farm in-		saving	45
come	8	Significance of saving	46
Fluctuations of cash farm income		Significance of investment	47
within major branches of produc-		Indicators of investment	48
tion	10	Investment and income	50
3. Prices and production as factors affect-		Investment and farm prices	50
ing fluctuations of cash farm income..	19	Investment and foreign trade	52
Summary	19	8. Factors affecting changes of investment,	
Fluctuations of income and prices..	19	and the role of money	53
Fluctuations of income and quantity	19	Summary	53
4. Trend and fluctuations of production		Quantity and value of investment ..	53
expenses and charges since 1910	21	Factors affecting changes of invest-	
Summary	21	ment	53
Significance of fluctuations in pro-		Role of money	55
duction expenses and charges	21	9. Conclusion: some implications for pub-	
Wage-rate fluctuations	21	lic economic policies and for individual	
Fluctuations in other productive		action	59
services	25	Agricultural programs and anticycli-	
Fluctuations in taxes and interest..	28	cal policies	59
5. Foreign demand as a factor in changes		Public versus private investment de-	
of cash farm income	30	cisions	59
Summary	30	Price structure	60
Value and quantity of total agricul-		Inflationary tendencies	60
tural exports	30	Two questionable public policies ..	60
Quantity of agricultural exports by		Subsidies to consumption or to in-	
major groups of commodities	31	vestment?	61
Value of agricultural exports and		The future of investment	63
cash farm income	32	In secular perspective	63
6. Domestic demand as a factor in changes		In the intermediate future	64
of cash farm income	36	The need for public policy	65
Summary	36	The goal of public policies	65
Levels of domestic demand	36	Fiscal and monetary policies	65
Number and composition of the agri-		Public debt	66
cultural population	37	Type of public-investment outlets..	67
Rate	37	Conservation policy	67
Time between harvest	39	Taxation policy	67
Income distribution	39	What the farmer can do himself ..	68
Land	41	Literature cited	70
Government	41	Range of figures and tabulations	71

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S. V. CIRIACY-WANTRUP²

1. INTRODUCTION

IT IS COMMONLY EMPHASIZED that the economic position of California agriculture is subject to special factors that do not operate, or operate to a much smaller extent, in other parts of the nation. Special conditions in climate, soils, topography, economic location, and history, and, accordingly, in types of products, yields, prices, and organization, put farming in California in a class by itself if compared with that in other states. Still, for California agriculture as a whole, as well as for its individual branches, changes in economic position are influenced greatly by major economic forces affecting farmers regardless of type of product and location. This study is concerned with such forces.

In order to explain the nature and significance of major economic forces, it appears best to analyze their operation in the past. On such a basis, obviously, no hard-and-fast forecasts about the future can be made. One may attempt, however, to help the reader recognize the direction and strength of present tendencies and appraise public economic policies designed to cope with them.

For this attempt it is useful to start the analysis before the first world war. Statistical data for individual states are rather unsatisfactory in volume and reliability before 1910. On the other hand, economic tendencies visible after World War II have important characteristics in common with those that were created through World War I. Although it cannot be expected that history will repeat itself, it is highly instructive to observe how the economic forces in which we are interested have operated during and after a major war.

In the historical analysis, conditions in California agriculture will be compared, as far as available data permit, with conditions in the United States excluding California. One objective of this study is to ascertain how far and in what way the economic position of California farming is related to that of

¹ Paper no. 121, the Giannini Foundation of Agricultural Economics; received for publication May 20, 1947.

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other agricultural regions of the country, to the nonagricultural national economy, and to foreign trade.

Changes in the economic position of California farmers will be studied in three stages: (1) with respect to gross income, (2) with respect to production expenses and charges, and (3) in relation to other parts of the national economy. This procedure enables separate analysis of the most important economic forces, and makes the best use of available statistical data. Extensive use will be made of graphs. By way of illustration, these graphs will help in understanding the often rather complex and controversial relations which will be discussed. The statistical data on which graphs and tabulations are based are explained on pages 71 to 76.

2. TREND AND FLUCTUATIONS OF CASH FARM INCOME SINCE 1910

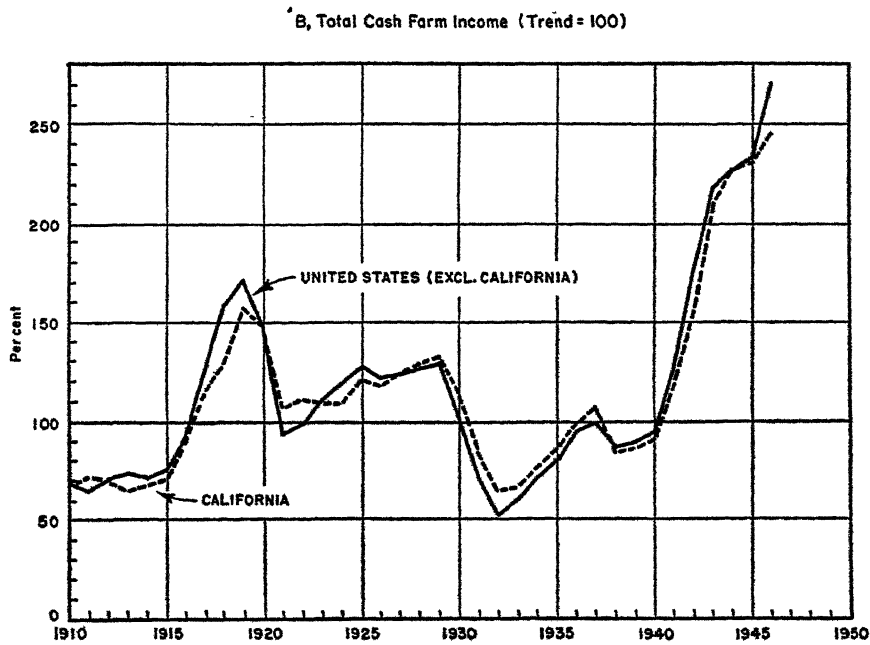
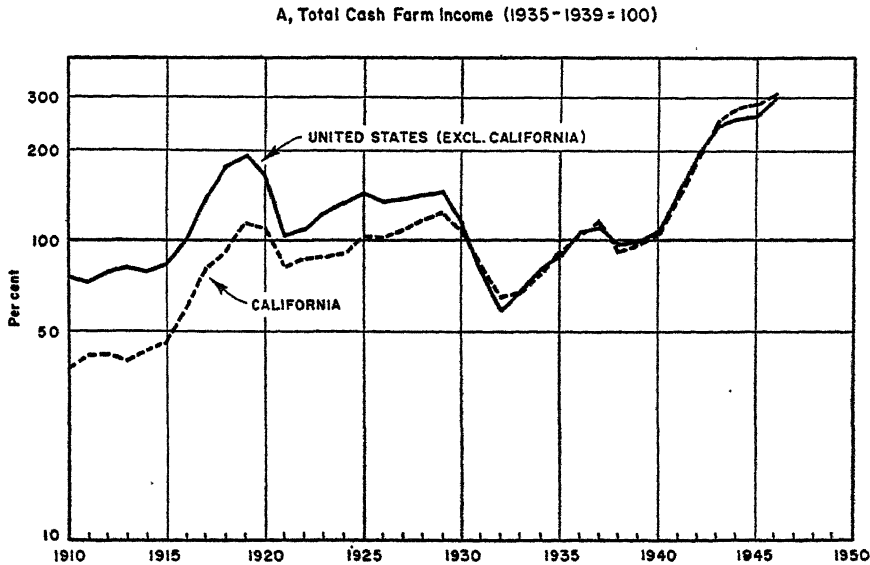
Summary. A comparison of cash farm income in California with that in the rest of the United States shows that: (1) Major economic fluctuations affect cash income of farmers in California in about the same way as that of farmers in the rest of the nation. (2) The effects are great in all major lines of production. (3) The effects are more important for aggregate changes of cash farm income than long-time growth (trend) and year-to-year variations in yields, acreage, and livestock production.

Changes of Cash Farm Income. Gross farm income is composed of cash income from farm marketings, of the value of home-consumed products, and of government payments (conservation payments, parity payments, production subsidies, and so on). The first source is by far the most important one; during the decade 1935 to 1944, when government payments began to play a role in American agriculture for the first time, 96.4 per cent of the gross income of California agriculture came from farm marketings, and only 1.8 per cent each from home consumption and from government payments. Moreover, the nature of major economic forces can most effectively be explained by confining the analysis to cash farm income. The quantity of home consumption is statistically unreliable, and its value is largely determined by the same forces that affect the value of farm marketings. Our discussion, therefore, must focus on cash farm income.

Changes in cash farm income from all sources (crops and livestock) in California and in the United States excluding California are graphically presented in figure 1. At one glance two observations come to mind in studying the upper figure (fig. 1, A): First, there is an upward long-time trend of both series. This trend is stronger for California than for the United States excluding California. Second, there are fluctuations which are closely similar in amplitude and direction for California and for the United States excluding California.

A third fact cannot be observed from the indices: The average cash income per person of the farm population is considerably higher for California than for the United States excluding California. During the period 1939-1944, for example, average cash farm income per person per year was \$1,844 in Cali-

FIG. 1. TOTAL CASH FARM INCOME FOR CALIFORNIA
AND THE UNITED STATES EXCLUDING CALIFORNIA



fornia and only \$464 in the United States excluding California. The most important factors which account for this difference in income levels may be considered with the factors that cause trend and fluctuations. Farmers and policy makers are less concerned with past and present levels of income than with the forces that may bring about changes of income in the future. Attention, therefore, must be focused on trend and fluctuations of cash farm income. First, we will consider trend.

Trend of Total Cash Farm Income. The difference between California and the United States excluding California in the rate of long-time increase of cash farm income was especially noticeable from 1910 to 1930, almost disappeared between 1930 and 1940, and showed a tendency to reassert itself thereafter (fig. 1, *A*). This difference and its changes over time correspond to differences in the growth rate of the farm population and farm employment (fig. 2). Until about 1930, California shows the signs of an expanding agriculture in a young country, whereas the United States excluding California exhibits the behavior of agriculture in a more mature industrial economy, that is, stationary or slowly declining farm population and decreasing agricultural employment. After 1930, California begins to follow the pattern which prevailed for some time in the United States excluding California.

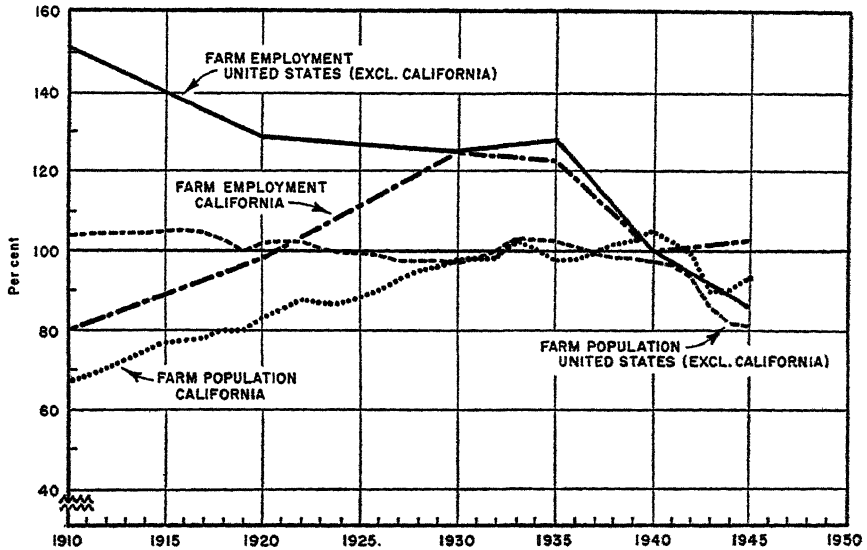
It may be noted that the long-time increases and decreases in the farm population are modified by cyclical movements (fig. 2). These are the effects of rural-urban and urban-rural migrations during prosperity and depression as well as of migration to California for other reasons—especially because of drought outside of California in the middle of the 1930's and because of the boom in Pacific Coast industries during World War II. California farm population is more influenced by these migrations than the farm population of the United States excluding California. The ratio of farm population in California to that in the United States excluding California exhibits, therefore, fluctuations around a trend which, in itself, corresponds to the trend in ratios of farm employment and cash income (fig. 2, *B*).

On the production side, the difference in growth rate (and changes over time in this difference) between California and the United States excluding California are connected with the shift (and changes over time in the rate of this shift) in California from dry farming to irrigation agriculture; from general field crops to fruits, nuts, and vegetables; and from range livestock (beef cattle, sheep) to dairying. Total acreage farmed and total cropland underwent only minor changes. Total acreage farmed was (in millions of acres): 1910, 27.9; 1920, 29.4; 1930, 30.4; 1940, 30.5; 1945, 35.1. Total cropland was (in millions of acres): 1910, 11.4; 1920, 11.9; 1930, 11.5; 1940, 12.9; 1945, 11.4. The Census of Agriculture of 1910 was less complete than succeeding ones. The real changes are, therefore, even smaller than those which are statistically indicated. The increase in total acreage farmed between 1940 and 1945 is due to the fact that more public grazing lands and Indian grazing lands are included; this increase does not necessarily mean that more land is used for agricultural purposes.

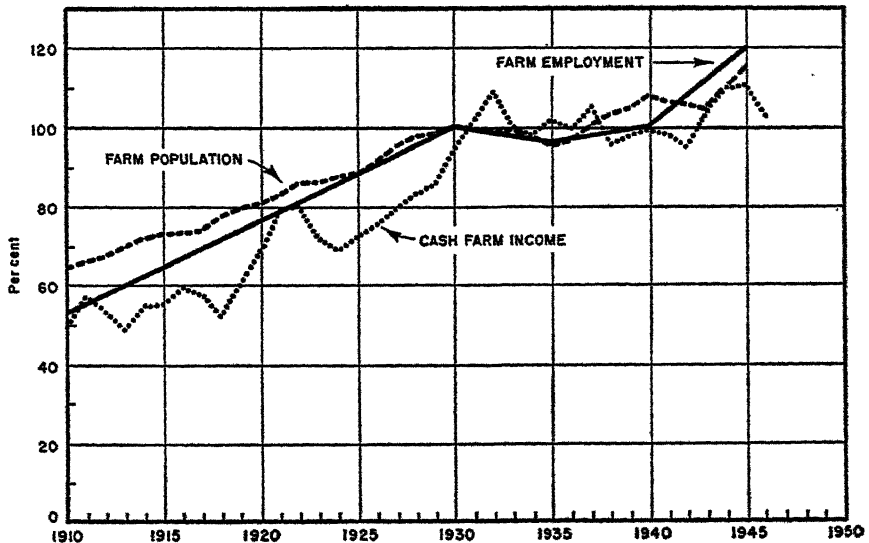
The shifts represented mainly a great increase in the intensity of California farming—that is, an increase in input and output per acre. Let us look somewhat closer at the nature of these shifts.

FIG. 2. GROWTH OF FARM POPULATION AND FARM EMPLOYMENT IN CALIFORNIA AND IN THE UNITED STATES EXCLUDING CALIFORNIA

A, Farm Population and Farm Employment for California and the United States Excluding California
 Farm Population (1936-1939 = 100)
 Farm Employment (1940 = 100)



B, Ratios of Indices of Farm Population, Farm Employment, and Cash Farm Income in California to Those in the United States Excluding California



On the basis of the Census of Irrigation, irrigated acreage in California increased 1,554,936 acres, or 58.4 per cent, between 1910 and 1920;^{*} 527,592 acres, or 12.5 per cent, between 1920 and 1930; and 322,936, or 6.8 per cent, between 1930 and 1940. These rates of increase and their changes by decades are highly significant for California cash farm income for two reasons: First, irrigated acreage is a large percentage of total cropland harvested. On the basis of the 1940 Census of Agriculture, this percentage was 65.4 per cent in California, against 4.3 per cent in the United States excluding California. Second—under nonhumid climates, at least—per-acre productivity is higher for irrigated than for nonirrigated land. On the basis of the 1930 Census of Irrigation (the 1940 census does not permit a similar calculation) irrigated cropland harvested in California produced \$126.05 of crops per acre; nonirrigated cropland harvested produced only \$29.58 per acre—a relation of approximately four to one. The ratio of per-acre productivity between all irrigated land and all nonirrigated land is doubtless even larger but cannot be ascertained statistically.

The greater importance of the irrigated acreage in California (than in the United States excluding California) is significant for the higher cash farm income per person in California which was observed above. Total cropland per person of the farm population is not much larger in California than in the United States excluding California (19.2 acres against 17.3 acres on the basis of the 1940 census). The difference is somewhat greater in total farm land (45.5 acres against 34.5 acres). However, the per-acre productivity of farm land other than cropland is small. On the other hand, there are (1940 census) 6.4 acres of irrigated land per person of the farm population in California against only 0.5 acres in the United States excluding California.

Changes in crop and livestock production are partly induced by expansion of irrigated acreage. Partly, therefore, changes over time in the rate of these changes correspond to the above changes in the rate of increase of irrigated acreage. Until the 1920's, changes of this kind were probably largely responsible for the growth of California cash farm income.

On the other hand, changes in crop and livestock production may take place within irrigation agriculture or (of smaller importance for California) within dry farming. Such changes, therefore, may proceed independently of an increase of irrigated acreage. The main factors in such changes are improvements in technology of production, processing, and marketing; more and better capital equipment; greater use of fertilizer; better soil-conservation practices; and improved varieties of plants and strains of animals. Since the 1920's this second type of change was probably more important for increases in California cash farm income than the first one.

Which one of these two types of change is more important, is significant for the relative growth rates of agriculture in California and in the United States excluding California in the future. California has no particular advantages with respect to the second type of shift. Improvements in technology are not confined to irrigation agriculture. Although the California farm population uses twice as much equipment per head as the farm population in the

* The statement made in the text about the Census of Agriculture of 1910 applies also to the Census of Irrigation of 1910—but possibly in a somewhat smaller degree.

United States excluding California (this fact is also significant in connection with the higher cash farm income per person of the California farm population which was observed above), California did not quite maintain this lead in recent decades. On the basis of the census, relative values of farm implements and machinery per person of the California farm population were (United States excluding California = 100) 226 in 1910; 237 in 1920; 209 in 1930; 204 in 1940; and 178 in 1945.⁴ In California, fertilizer use is burdened with higher transportation charges than in the East. Soil-conservation practices will probably increase production per acre more in other parts of the country than in California. Improved varieties of plants and strains of animals and better processing and marketing facilities are also of greater importance for states which in these respects are less advanced than California.

Changes in the rate and type of long-time growth of California agriculture are of considerable interest for the analysis of economic fluctuations. A clear differentiation between the influence of long-time growth and of economic fluctuations is necessary for three reasons:

1. The effect of long-time growth applies to aggregate cash income of agriculture but does not usually apply to all branches of production or to all individual farmers. For example, long-time growth in California cash farm income cushioned the decline after 1920. However, some branches of production and many farmers did not participate in this effect, which was, as we know, mainly related to the increase in irrigation agriculture. Economic fluctuations, on the other hand, usually influence all branches of production and all commercial farmers—as shown below.

2. The relative importance of long-time growth and of economic fluctuations may change over time. The cushioning effect just mentioned applied to the economic depression after 1920, but, because of changes in growth rates already explained, much less to the one after 1930. What about the future? There are some indications that the future long-time growth rate of California cash farm income as a whole, at least for one decade, will be closer to the rate prevailing between 1920 and 1930 than to that between 1930 and 1940. Large new public irrigation developments are in construction, or have been authorized, or are contemplated. A rapidly growing nonagricultural population in California provides incentives on the demand side toward further intensification of agriculture. In the more distant future, on the other hand, the growth of California agriculture and of its economy generally is limited by one important factor: the availability of water. How far it will be technically and economically possible to overcome this limitation cannot be foreseen at this time.

3. A differentiation between the influence of long-time growth and of economic fluctuations is necessary because the latter are much more important for the individual farmer. This is true not only because their influence usually affects all farmers. More important is the fact that the magnitude of their influence per unit of time (per year) is much greater. The long-time average growth of California cash farm income from 1910 to 1941 was 3.4 per cent per year. Economic fluctuations, on the other hand, frequently caused deviations

⁴ Census data on value of farm equipment are by their nature not very reliable. However, this difficulty is minimized if only ratios are used, as in the text.

from this long-time growth of 20 per cent or more from year to year.⁵ For this reason our present study must emphasize the economic forces that cause fluctuations of cash farm income rather than its long-time growth.

In order to focus on the influence of economic fluctuations upon cash farm income, the influence of long-time growth will be taken into account throughout this study by straight-line trends computed for the years 1910 to 1941. Thus, these trends are not influenced by the war and first postwar years. The high values for these years, at the very end of our period of analysis, would have exercised great statistical influence upon the slope of the trends. For reasons which will become apparent below, such a statistical influence seems undesirable because future developments (if they could be taken into account in trend computation) will probably counterbalance the influence of recent years upon the slope of the trends.

In this study, straight-line arithmetic trends computed by the method of least squares are used throughout. Theoretically, logarithmic trends are preferable for most series related to production—for example, cash farm income; for price series arithmetic trends are generally preferable. For comparison, a few experiments with straight-line logarithmic and straight-line arithmetic trends were undertaken. Practically, the difference between the two types of trend was insignificant for the period used for trend computation (1910–1941). It was greater for the period of analysis (1910–1946). It was felt, however, that straight-line arithmetic trends were most easily understood, and that use of different types of trend for different series and different periods might be confusing.

With respect to California, it may be well to repeat that the trends thus computed understate the growth rate of cash farm income from 1910 to 1920, correspond approximately to the growth rate from 1920 to 1930, and overstate the growth rates from 1930 to 1940. It will be shown later that changes in growth rate are related to economic fluctuations. The fact that these changes in growth rate are not taken into account by our trends, is, therefore, no bias for the purpose of our analysis. On the other hand, to take changes in growth rate into account through trends computed by seven- or nine-year moving averages would have restricted our period of analysis too severely and eliminated a part of the fluctuations in which we are interested.

Fluctuations of Total Cash Farm Income. Fluctuations of total cash farm income for California and the United States excluding California are shown as relative deviations from trend in figure 1, *B*. It is apparent that there are only small differences in the severity of these fluctuations between California and the United States excluding California. Fluctuations in California tend to be slightly less than in the United States excluding California. This is caused by the relatively (compared with the United States excluding California) smaller importance for California of staple crops and hogs, and the greater importance of vegetables. It will be shown presently that fluctuations in the former branches of production tend to be more violent than in the latter. Generally, however, the close correspondence of fluctuations of cash farm income between California and the United States excluding California is

⁵ These deviations are calculated on the basis of yearly averages. On the basis of monthly averages, deviations from year to year would be even greater.

remarkable. This correspondence exists in spite of the differences in the income per person already referred to, and the great differences in the composition of total cash farm income within individual branches of production.

It is generally believed that California agriculture is more immune to economic fluctuations (compared with agriculture in the rest of the country) than seems to be indicated by our findings. Such opinion is based largely on the well-known fact that California farm real estate values per acre showed a less precipitous decline after 1920 than such values in the United States as a whole (Regan, Johnson, and Clarenbach, 1945, table 1).⁶ It is necessary, therefore, to scrutinize this apparent contradiction.

Farm real estate values are averages by crop reporting districts. These averages are not weighted by type and size of farm. The state average is obtained by combining the district averages through weighting by total land in farms within districts. Before 1934 no attempt was made to differentiate between dry-farmed and irrigated land. Crop reporter's estimates are strongly influenced by sales prices of transfers that come to their attention. Because of these methods of computation, changes in the state average are decisively affected by a rapid shift from dry farming to irrigation agriculture. It was shown above that such a shift was still in full swing for California around 1920. This shift increases greatly the value of individual transfers: in 1945 the average value per acre in selected counties, seven western states, was \$21.26 for grazing land, \$111.83 for cultivated nonirrigated lands, and \$384.82 for irrigated land (Stonecipher and Dunn, 1946, table 2).⁷ In addition, such a shift increases the weight of irrigated land in the average because irrigated farms are smaller and change hands more often. On the basis of the 1920 Census of Irrigation, the average size of irrigated farms in California was 62.6 acres; the average size of nonirrigated farms was 500.1 acres. The corresponding figures for the 1930 Census of Irrigation were 55.3 acres and 515.0 acres. After 1930 this influence was much smaller because, as we know, the expansion of irrigated land had slowed down, and because (since 1934) the statistically essential differentiation between major types of land was made in computing averages. From then on, changes in California real estate values per acre are close in direction and amplitude to such changes for the country as a whole. This parallelism would probably appear earlier also if proper statistics were available. The census cannot be used for testing because suitable data appear only in 1930 and 1940 censuses.

Besides the parallelism of major fluctuations of cash farm income in California and the United States excluding California, their quantitative significance (that is, in terms of amplitude and time) deserves emphasis. The following low and high annual averages, in per cent of trend, indicate the amplitude of major fluctuations since 1914:⁸

	1914	1919	1921	1929	1932	1946
California	68	157	107	132	65	246
Other states	70	172	94	130	53	270

⁶ See "Literature Cited" for citations, referred to in the text by author and date.

⁷ It may be noted that the value ratio of irrigated land to cultivated nonirrigated land is slightly less than 4:1. This situation agrees well with the per-acre productivity ratios computed above.

⁸ As already implied (footnote 5), high and low points would indicate considerably greater amplitude of fluctuations if daily or monthly averages had been used instead of annual.

The farmer is chiefly concerned with these major fluctuations, rather than long-time trend and minor year-to-year oscillations.

In order to analyze the forces causing these fluctuations of total cash farm income, it is important to ascertain whether and to what extent the major branches of California agriculture were affected.

Fluctuations of Cash Farm Income within Major Branches of Production. For our purposes the following four branches of production may be differentiated: (1) livestock and livestock products; (2) fruits and nuts; (3) vegetables; (4) field crops. Cash farm income for these branches is shown in figure 3.

It is evident that all branches show characteristics of trend similar to those discussed above for total cash farm income. However, the difference in the rates of long-time growth between California and the United States excluding California is not so noticeable in livestock and livestock products as in the other branches. The livestock industry was less affected than crop production by the shifts to irrigation agriculture and to more intensive land use with which, as we know, the stronger long-time growth of California agriculture was connected. As in total cash farm income, absolute and relative (as compared with the United States excluding California) growth rates in California are largest from 1910 to 1920, somewhat less from 1920 to 1930, almost disappeared between 1930 and 1940, and show an increase since then.

For studying fluctuations of cash farm income within branches of production, long-time growth was taken into account by the same method explained above for total cash farm income. The results are shown in figure 4. It can be seen that for all series, major fluctuations are similar for California and for the United States excluding California. Within the individual sets, however, some considerable differences exist. It is advisable, therefore, to discuss the four sets individually.

Fluctuations in cash farm income from livestock and livestock products show an amplitude similar to that of total cash farm income and a similar close correspondence between California and the United States excluding California, except a smaller expansion in California during the two war periods (fig. 4, A). This exception may be explained as follows: California's most important feed base—the natural range—is rather fixed, and is more suited for beef cattle, sheep, and dairying than for hogs. For the United States excluding California, on the other hand, hog production based on home-grown grain is much more important. These differences are reflected in the data in the table on the next page. Biologically, and on account of the feed base, the hog enterprise permits a quicker and larger expansion under the stimulus of high prices than other livestock enterprises, except poultry. California's important poultry industry, however, was handicapped during wartime because it is largely based on imported feed. One may also note (fig. 4, A) that the well-known production cycles in livestock, especially in cattle, hogs, and sheep, had, as compared with the economic fluctuations in which we are interested here, only a small effect upon changes in aggregate income. This effect is noticeable mainly by causing slight differences between our two series during 1927–28 and 1936–37. In both periods general economic conditions were relatively stable. During these periods the peak of slaughtering in the hog cycle was espe-

cially high (and the trough of prices and incomes especially low) because of extreme fluctuations of corn yield. As already mentioned, California is less affected by the hog cycle than the United States excluding California, because of the smaller importance of hogs and of corn yield for production fluctuations. The relative importance of cash farm income from the various livestock industries in 1926 to 1929,* expressed in per cent of income from all livestock and livestock products, was as follows:

	California, per cent	United States excluding California, per cent
Dairy products	34.9	28.9
Cattle, calves	27.0	24.0
Eggs and chickens	21.2	17.5
Sheep, lambs, wool	9.4	5.1
Hogs	5.1	22.8
Other	2.4	1.7
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All livestock and livestock products	100.0	100.0

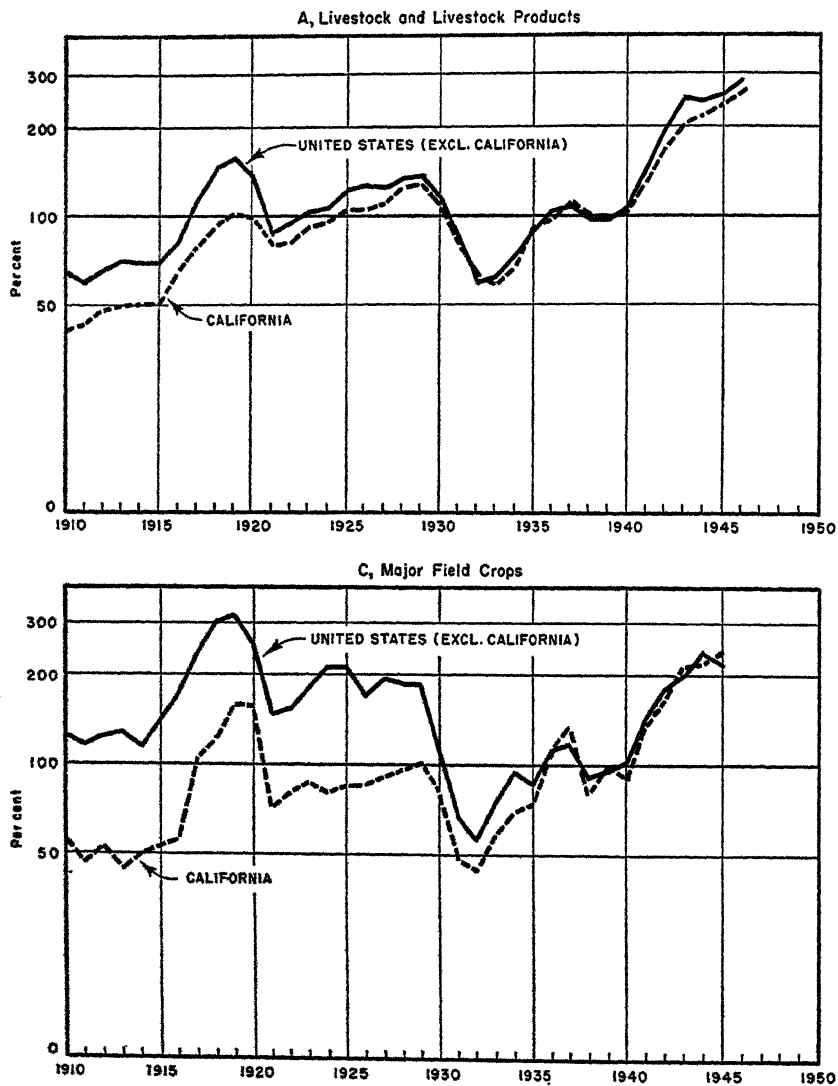
The various fruit and nut enterprises in California differ greatly in importance from those in the United States excluding California. The following data show the relative importance of cash farm income from the various fruits and nuts in 1926 to 1929, expressed in per cent of income from all fruits and nuts:

	California, per cent	United States excluding California, per cent
Oranges	37.0	7.5
Grapes	17.7	2.9
Lemons	8.8	0.0
Prunes	6.6	1.2
Nuts	6.1	2.4
Peaches	6.1	10.1
Apricots	4.4	0.1
Pears	3.8	3.6
Apples	3.0	41.6
Strawberries	1.5	14.2
Cherries	1.1	2.9
Grapefruit	0.7	5.3
Others	3.2	8.2
	<hr/>	<hr/>
All fruits and nuts	100.0	100.0

In fruits and nuts, furthermore, variations in yields between years and between regions are especially great. In spite of these conditions, the correspondence between income fluctuations in California and in the United States excluding California is rather close both in direction and amplitude (fig. 4, *B*).

* The four-year period 1926 to 1929 is chosen for this and the following tabulations in this section because it is the center of the period 1910 to 1945; is the beginning of a period of better statistical data; and is, comparatively, a period of economic stability.

FIG. 3. CASH FARM INCOME BY MAJOR BRANCHES OF PRODUCTION FOR



CALIFORNIA AND THE UNITED STATES EXCLUDING CALIFORNIA (1935-1939 = 100)

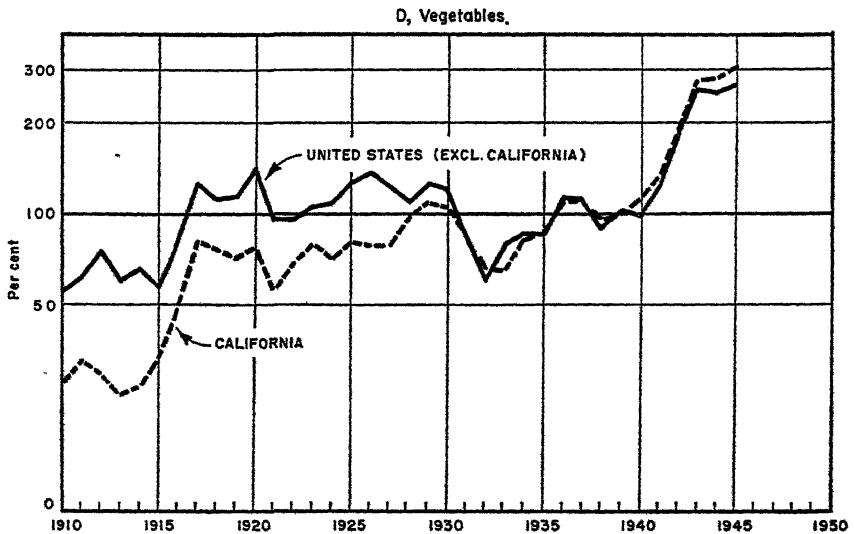
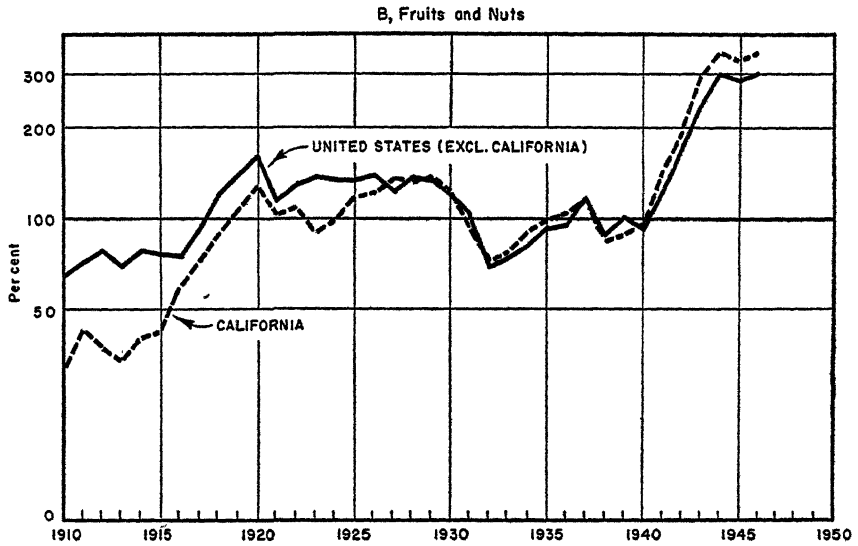
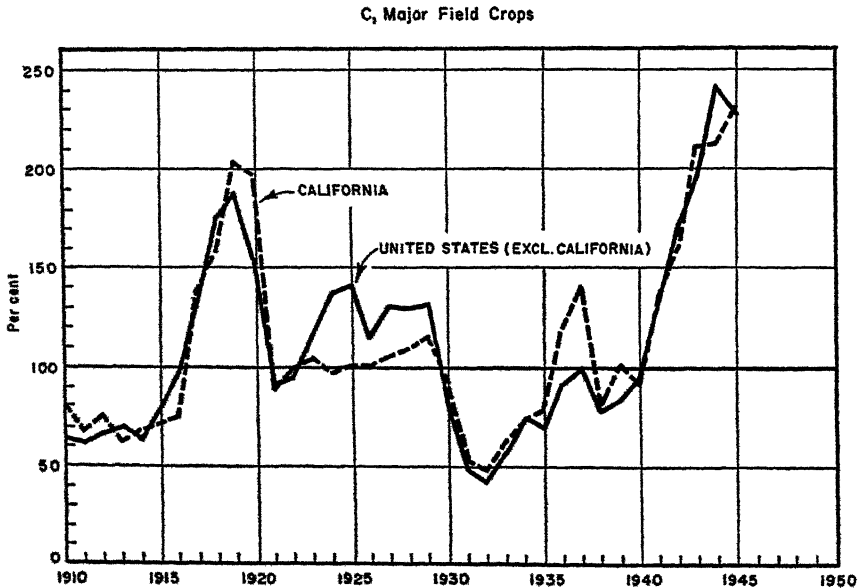
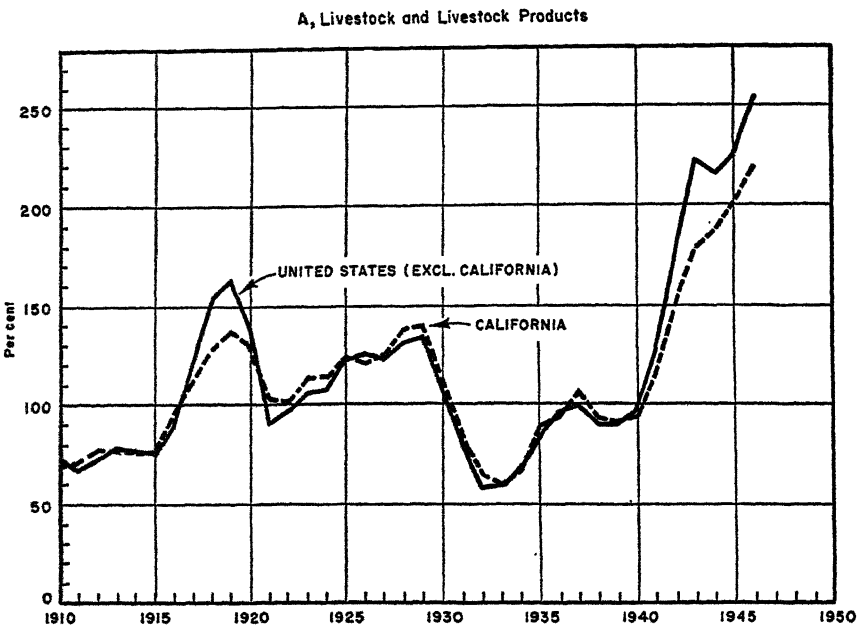
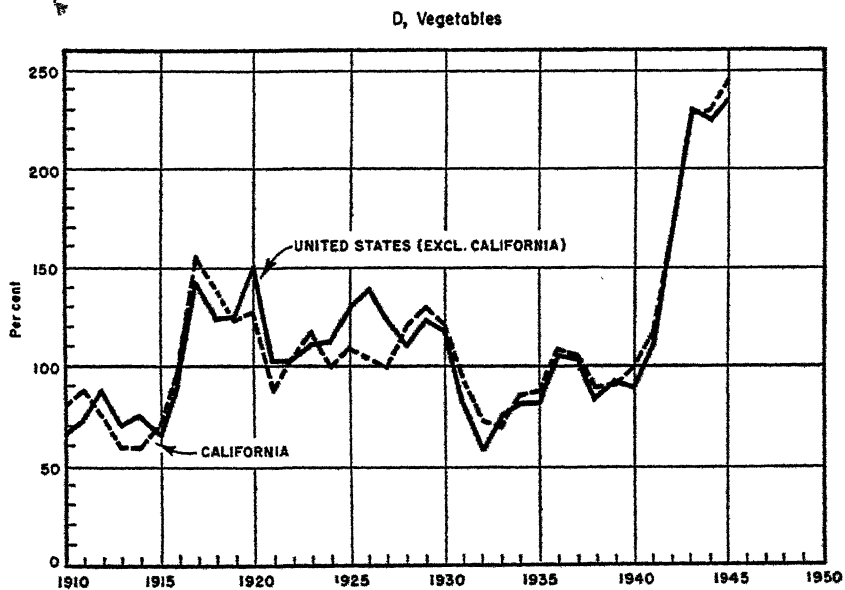
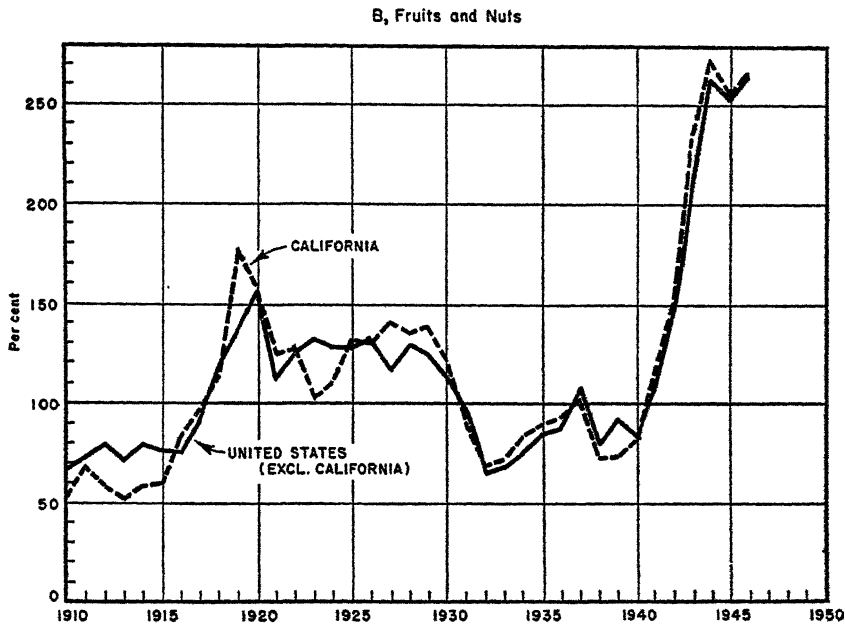


FIG. 4. CASH FARM INCOME BY MAJOR BRANCHES OF PRODUCTION FOR



CALIFORNIA AND THE UNITED STATES EXCLUDING CALIFORNIA (TREND = 100)



The major discrepancy lies in the behavior of the two series between 1919 and 1923. This discrepancy may be explained largely by relatively good income from apples, which have great weight in the United States excluding California, but small weight in California, and by poor income from grapes (prohibition became effective in January, 1920), the relative weights of which are the opposite from those of apples. Fluctuations between 1910 and 1930 were more violent in California than in the United States excluding California. This is noteworthy because students who contend that California was more immune to economic fluctuation in the beginning of the 1920's usually point to California's specialities as an explanation.

Income from major field crops in the United States excluding California is heavily weighted by cotton, whereas hay is relatively more important for California. The relative importance of cash farm income from the various field crops in 1926 to 1929, expressed in per cent of income from all major field crops, is shown in the following data:

	California, per cent	United States excluding California, per cent
Cotton and cottonseed.....	25.0	48.6
Wheat	21.4	27.5
Hay	19.7	4.6
Barley	18.6	1.2
Rice	12.4	1.1
Oats	1.6	3.8
Corn	1.3	13.2
All major field crops.....	100.0	100.0

The differences between the two series, therefore, are largely explained by differences in the prices (and to a smaller degree in yields) of cotton and hay (fig. 4, *C*). This is true, for example, for the greater and more erratic increase in the United States series between 1921 and 1929 and the especially good showing of California during the drought period in the second half of the 1930's. The great differences in the relative weight of individual grains between the two series are of no fundamental importance for price movements; grain prices, as a whole, move in close unison because of substitution. Differences in yields, however, were important during the middle of the 1930's. California was less affected by the drought; rice is grown under irrigation, and is of much greater importance in California than in the United States excluding California. Beyond these differences, figure 4, *C*, reveals clearly that the income from major field crops shows the same cyclical fluctuation in direction and amplitude in California as in the United States excluding California. Fluctuations around 1920 were at least as violent in California as in the rest of the country. The direction of fluctuations corresponds closely with those observed in the two previous groups of commodities; their amplitude, however, is greater.

In contrast to the income from "staples" just discussed, income from vegetables shows less amplitude in cyclical fluctuations—although fluctuations are clearly present and correspond in direction to those already noted (fig. 4, *D*).

Year-to-year variations in yields and acreage and their effects upon yearly and seasonal price movements are of greater significance (in relation to cyclical fluctuations) for vegetables than for other groups of products. These variations are frequently different in direction between California and the United States excluding California. Furthermore, great differences exist in the relative importance of individual vegetables between the two regions, as shown by the following data on cash farm income from various vegetables in 1926 to 1929, expressed in per cent of income from all vegetables:

	California, per cent	United States excluding California, per cent
Dry edible beans.....	19.5	6.6
Lettuce	18.7	2.8
Cantaloupes	11.0	2.2
Asparagus	7.9	1.3
Potatoes	7.4	41.7
Tomatoes	7.4	9.7
Peas, green	4.7	3.6
Celery	3.5	2.7
Onions	3.2	4.9
Cauliflower	2.9	0.6
Artichokes	2.5	0.0
Sweet potatoes	1.4	4.8
Beans, snap	1.2	3.7
Watermelons	1.2	2.3
Cabbage	0.9	4.5
Cucumbers	0.7	2.3
Others ¹⁰	5.9	6.3
All vegetables.....	100.0	100.0

The United States series is heavily weighted with potatoes, which have great yearly variations in yield and acreage. For California, on the other hand, income from such highly perishable commodities as lettuce, cantaloupes, and asparagus looms large. Furthermore, California's cash income from vegetables is more affected by beans than that of the United States excluding California. The differences between the two series during and shortly after World War I are largely explained by the latter factor.



We have seen that the major economic fluctuations affect the cash income of the California farmer much as they affect the income of farmers in the rest of the nation; that all major types of production are affected; and that their aggregate effects are greater on cash income than are those of trend or changes caused by harvest fluctuations and cycles in livestock production. Our next question, therefore, is: What factors cause major economic fluctuations?

¹⁰ "Others" includes, for California, carrots, Honey Ball melons, Honey Dew melons, pimientos, green peppers, and spinach; for the United States excluding California, carrots, Honey Ball melons, Honey Dew melons, pimientos, green peppers, spinach, lima beans, sweet corn, eggplant, beets, escarole, and kale.

FIG. 5. CASH FARM INCOME, PRICES RECEIVED BY FARMERS, AND QUANTITY OF FARM MARKETINGS FOR THE UNITED STATES (TREND = 100)

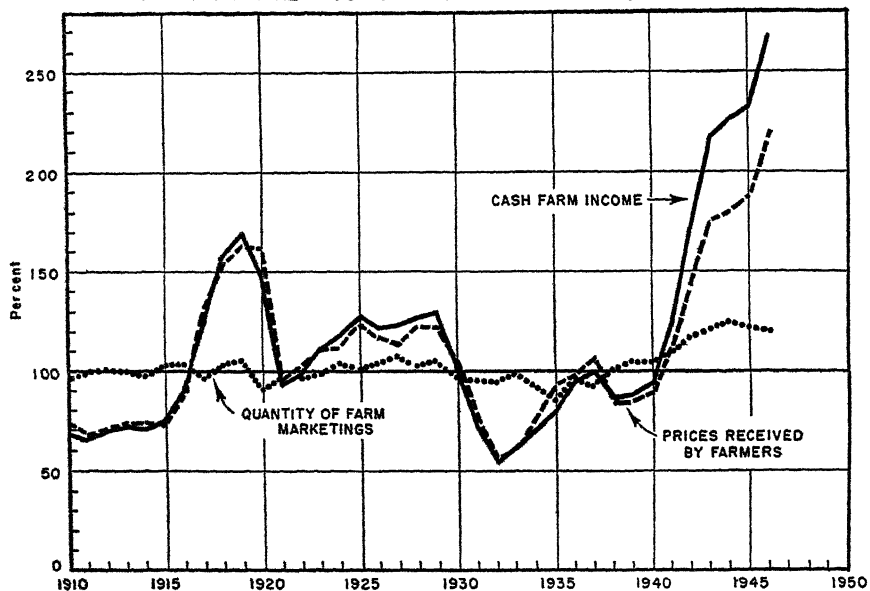
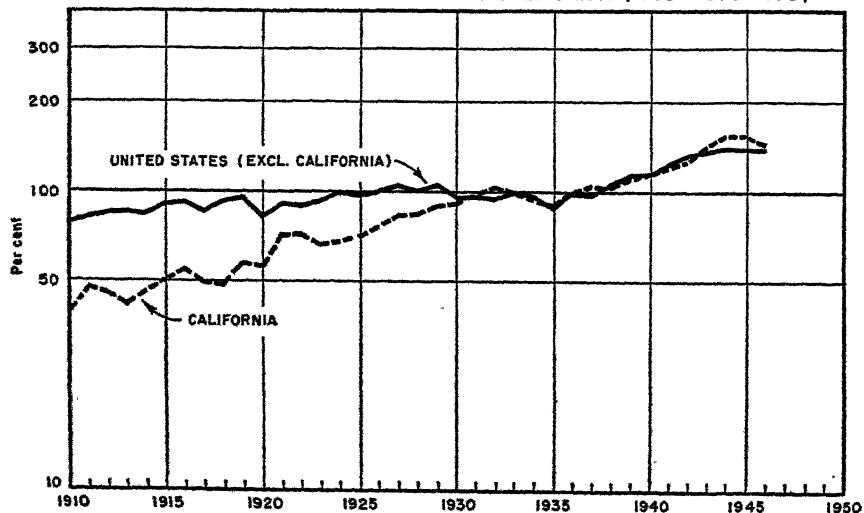


FIG. 6. QUANTITY OF TOTAL FARM MARKETINGS FOR CALIFORNIA AND THE UNITED STATES EXCLUDING CALIFORNIA (1935 - 1939 = 100)



3. PRICES AND PRODUCTION AS FACTORS AFFECTING FLUCTUATIONS OF CASH FARM INCOME

Summary. Major fluctuations of cash farm income for California and for the United States excluding California are largely price and not production phenomena. Quantities of farm marketings and production are rather stable, both for California and the United States excluding California. The rate of long-time growth in these quantities gives support to the method by which this rate was taken into account in the preceding section. Deviations from the rate of long-time growth (downward between 1929 and 1935 and upward between 1935 and 1946) do not account for major price changes. Price changes, on the other hand, appear to have influenced production in the same direction.

Fluctuations of Income and Prices. Cash income from farm marketings has two components: price and quantity. Although the latter is not identical with production, for brevity's sake, the two terms may be used interchangeably here. As indicated in the preceding section, human consumption by farm families of home-produced commodities is small in relation to marketings. Home production of feed, draft-power, and other services finds sufficient expression, at least for the purposes of this study, in the quantity of marketings.

If fluctuations in cash farm income and in prices are compared (fig. 5), it is at once apparent that the former are mainly price and not production phenomena. To be sure, differences in the two series are present. For example, the cash income series is considerably above the price series during World War II. During this period price increases were dampened by government controls. The war effort in production, on the other hand, was aided by good harvests, by depletion of soil fertility previously accumulated through the soil-conservation program, and by technological improvements—especially by greater use of fertilizers and improved plant and livestock varieties. The drought period in the middle 1930's caused another slight divergence of the two series. However, in the main, fluctuations of cash farm income are explained in direction as well as amplitude by fluctuations of prices.

Fluctuations of Income and Quantity. It can be expected from the interrelations just observed that the second component of gross income—namely, quantity of production and of marketings—is in the aggregate a relatively (compared with prices) stable factor (fig. 5). This is also borne out for California and the United States excluding California by the quantity indices presented in figure 6. These series were obtained by deflating the cash-income series (fig. 1) by the index of prices received by farmers in the United States. Theoretically, use of a common deflator for both series is not desirable, but was necessitated by the absence of an adequate farm price index for California. Practically, the bias involved is not likely to be material—as may be concluded from the relations already observed (figs. 1 and 5). The results are in good agreement with production indices computed for the United States and the

Pacific Coast states by the Department of Agriculture (Barton and Cooper, 1945). California production weights heavily in the total production of the Pacific Coast states.

The long-time upward trend and the differences in slope of this trend between California and the United States excluding California are almost identical for cash income and quantity marketed (figs. 1 and 6). The differences between decades in rates of long-time growth, which were discussed in the preceding section, are clearly noticeable in the quantity series. In general, however, the series support the rationale for computing a straight-line trend both for production and cash farm income. This is especially true because the strong upswing in production during World War II must probably be somewhat discounted in its significance for long-time trend. It has already been mentioned that this upswing was aided by good harvests, by depletion of soil fertility, and by better varieties of plants and strains of animals; these factors cannot be expected to operate to the same extent in the future. The decrease in the middle of the 1930's, which is more evident in the United States excluding California than in California, was largely due to the drought.

It may be noted that the period during which production rose faster than a straight long-time trend, that is, between 1935 and 1946, was also a period of rising prices. When prices declined between 1929 and 1935, the increase of production was markedly retarded, especially in the United States excluding California. Likewise, price fluctuations before 1929 do not correspond to fluctuations of production in the opposite direction. There is no indication, therefore, that variations in the volume of production were the cause of major fluctuations in prices. On the other hand, there is some indication that major swings in prices caused changes in the volume of production in the same direction.

Prices of farm products in this country are related to quantities marketed domestically *and* to marketings by foreign producers, as far as the latter influence international trade. Space does not permit a detailed review of quantities of agricultural products marketed in trading nations other than the United States. It has been shown elsewhere (Ciriacy-Wantrup, 1936, 1938a, 1938b) that our findings with respect to the relatively great stability of aggregate quantities of farm marketings and with respect to the parallelism between relatively small fluctuations of quantities and relatively great fluctuations of price, which were illustrated in figures 5 and 6 for the United States, hold also, and possibly more so, for the world's trading nations as a whole. In a later section (section 5), we will return to the relations between farm prices and cash farm income in the United States on one side and foreign trade on the other.



In this section we have seen that fluctuations of cash farm income are largely price and not production phenomena. The next step in our analysis is to determine whether prices of productive services and charges show fluctuations similar to those of farm prices and cash farm income. It will then be possible to draw conclusions with respect to changes of the farmer's fortunes during economic fluctuations.

4. TREND AND FLUCTUATIONS OF PRODUCTION EXPENSES AND CHARGES SINCE 1910

Summary. Price fluctuations, which were shown responsible for fluctuations in cash farm income (sections 2 and 3), appear also in fluctuations of farm wage rates and of prices of commodities used in production. These latter fluctuations, however, partly lag behind the former (as in the case of wage rates and farm-machinery prices) or show a considerably smaller amplitude (as in the case of prices of manufactured products in general). Real estate taxes and mortgage-interest burden exhibit even more lag and even smaller amplitude of fluctuations than production expenses. The mere fact of general price fluctuations, therefore, changes the economic position of farmers decisively. During the upswing the economic position of farmers tends to improve, during the downswing, to worsen.

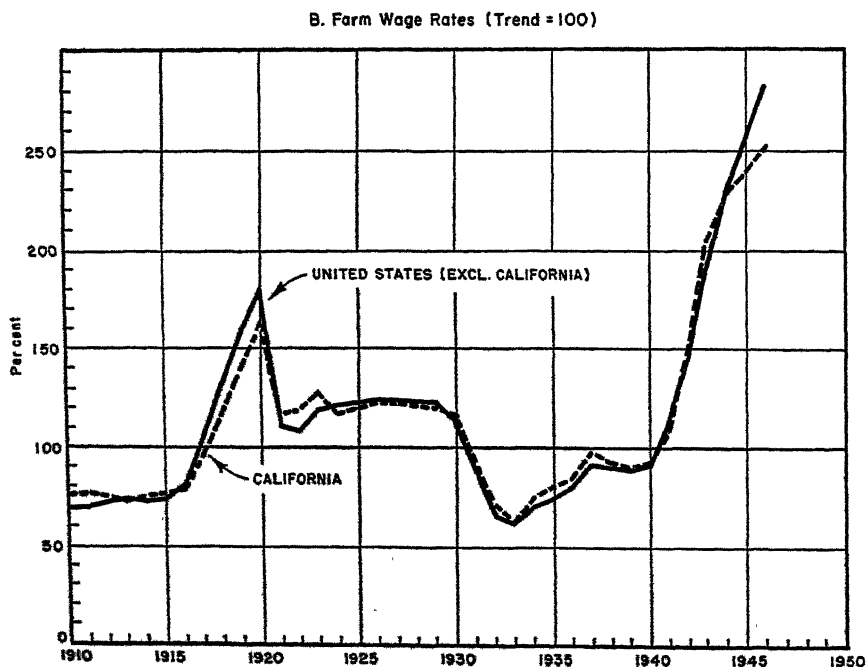
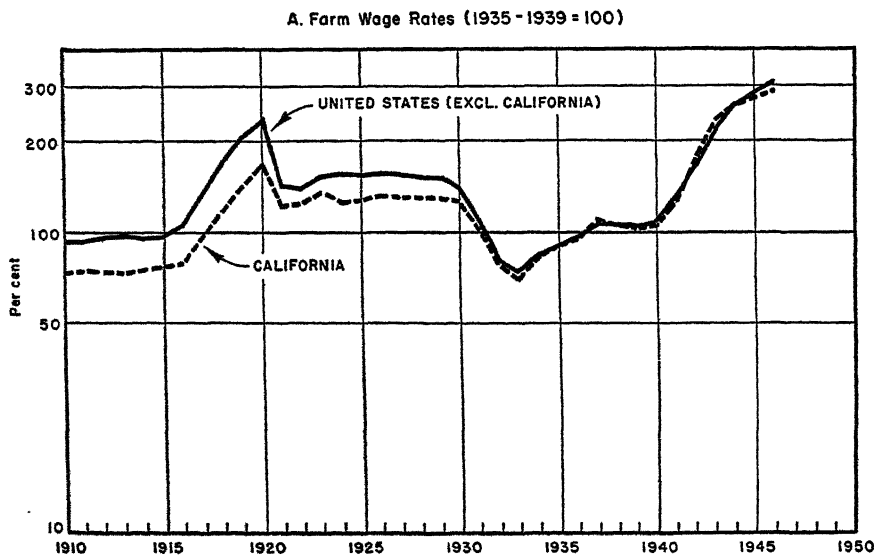
Significance of Fluctuations in Production Expenses and Charges. If prices of productive services and charges do not show the same fluctuations in direction or amplitude as prices received, the economic position of farmers will change in the course of price fluctuations.

The money net income of farmers would change in the course of economic fluctuations even if prices of productive services are assumed to change at exactly the same time and rate as prices received, and other factors (such as shifts of demand between products, changes of technology, and weather influences) are assumed unchanged. During the upswing, net income would increase, and during the downswing decrease, provided gross income is greater than expenses. However, such changes of net income would be of minor importance for the economic position of farmers because the purchasing power of net income would not be different than before if *all* prices in the economy (not only prices of productive services and of products) change at the same rate.

The changes in economic position we have in mind here are practically and analytically much more important. Our first objective is to point out these changes. Our second objective is to ascertain whether the extent and character of price fluctuations give any clues about their causes; to clarify these causes is the objective of the subsequent sections. Do price fluctuations extend also to wage rates? If so, how do timing and amplitude of fluctuations in wage rates compare with those in prices? Are there characteristic differences in amplitude of price fluctuations within various groups of commodities used in agricultural production? If so, what explains these differences? What is the relation between price fluctuations and those expenses of agriculture that are not prices—for example, the interest burden and the tax load?

Wage-Rate Fluctuations. Fluctuations in farm wage rates (fig. 7) are of special significance; for labor is the most important of production expenses incurred by California farmers. That this expense is relatively more important in California than in the United States excluding California is shown by the

FIG. 7. FARM WAGE RATES (PER DAY WITHOUT BOARD) FOR CALIFORNIA
AND THE UNITED STATES EXCLUDING CALIFORNIA



following data, giving percentage distribution of production expenses and charges for the period 1939 to 1944:

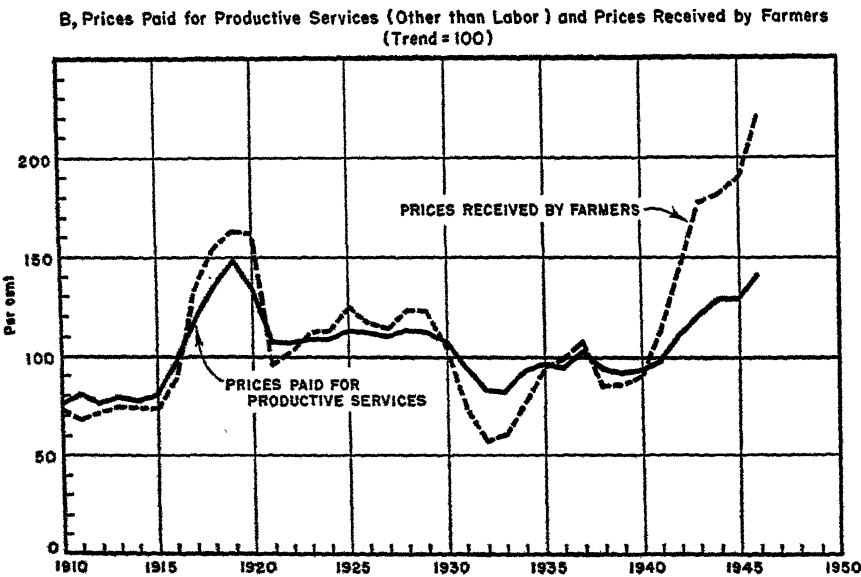
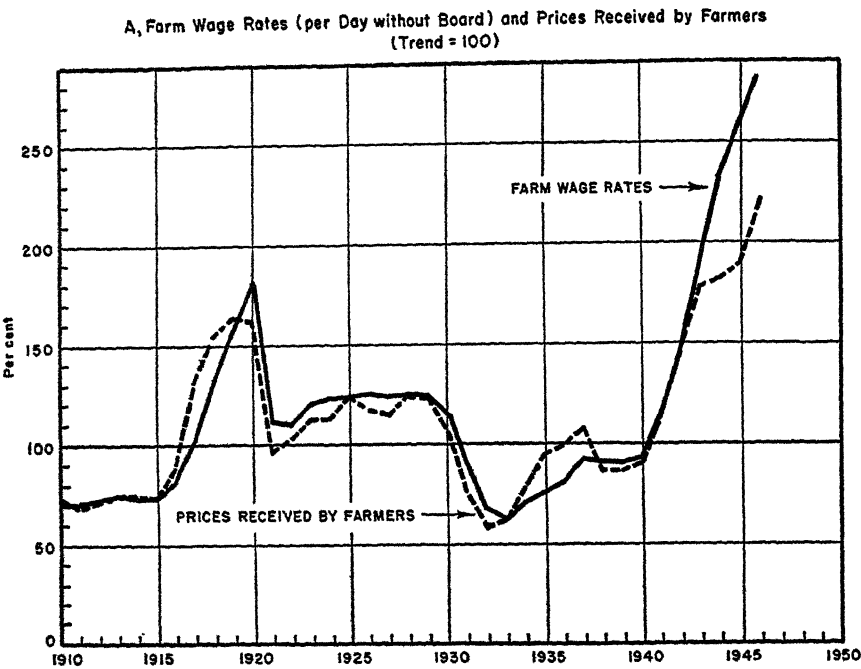
	California, per cent	United States excluding California, per cent
Hired labor	33.3	16.0
Feed purchased	16.0	16.8
Livestock purchased	6.1	8.7
Cost of operating motor vehicles....	5.9	8.3
Taxes	4.5	5.5
Buildings (maintenance or depreciation)	3.1	7.3
Farm-mortgage interest	2.8	3.4
Motor vehicles (maintenance or depreciation)	2.7	4.8
Machinery and equipment (maintenance or depreciation)	2.1	4.2
Fertilizer and lime	1.7	4.3
Miscellaneous	21.8	20.7
<hr/>		
Total production expenses and charges	100.0	100.0

There is considerable difference in absolute level of wage rates between California and the United States excluding California. Farm wage rates per day without board for the six years 1939 to 1944 (chosen for comparability in time with the above percentage-distribution data) averaged \$4.61 for California and \$2.46 for the United States.¹¹ Magnitude and causes of these differences correspond to similar ones discussed above in connection with cash farm income per person of the farm population. In spite of these great differences in level, and in spite of some differences in the slope of trend¹² during the first three decades of our period, fluctuations of wage rates in California were closely similar to those of the United States as a whole. It may be noted that the slight difference between the two series in the amplitude of movement from 1917 to 1921 corresponds to the differences between the two cash farm income series during the same period (fig. 1, p. 3). The same correspondence holds also for other periods when the differences between the California and the United States series were less conspicuous. This observation is important because (in the absence of adequate price data for California mentioned above) we must confine our comparison between fluctuations of wages and prices to the United States. However, since fluctuations in gross farm income are largely fluctuations in prices (fig. 5), and are closely similar for California and the United States excluding California (fig. 1), the relation between wage rates and price fluctuations for the United States as a whole (fig. 8, A) can be expected to hold also for California separately.

¹¹ Unfortunately an average for the United States excluding California is not available.

¹² For California $b = +0.270$; for the United States $b = -0.297$. Inclusion of the war years 1942 to 1945 would render the slope of the United States trend also positive. A difference in slope, however, would remain. This difference would be even greater if it had been possible to use data for the United States excluding California.

FIG. 8. PRICES RECEIVED AND PRICES PAID BY FARMERS, UNITED STATES



It is apparent from figure 8, *A*, that wage-rate fluctuations have followed price fluctuations with a slight lag, but with approximately the same amplitude. An exception is the period of World War II, when wage rates far outstripped prices. The causes for this exception are differences in public policies with respect to price and wage control. The flexibility of wage rates would have appeared even greater if data relating to rates with board had been used. Rates without board, however, are of greater significance for California agriculture. In industrial countries like the United States, the greater flexibility of farm wage rates—if compared with industrial wage rates—is related to less rigid unionization of the agricultural labor market, to the greater importance of payment in kind, and, particularly, to rural-urban and urban-rural migration during economic fluctuations. It has already been mentioned that California's farm population was considerably influenced by these migrations (section 2).

Wage rates change the economic position of farmers in the course of price fluctuations mainly through lag rather than through rigidity. The lag in the movement of wage rates behind that of prices received tends to impair the economic position of farmers in the beginning of price decreases, and to improve it in the beginning of price increases. On the other hand, the flexibility of wage rates for farm labor (together with the flexibility of remuneration under which the operator and his family work) is one of the reasons for the great stability of agricultural production which was noted above (fig. 6).

Fluctuations in Other Productive Services. In considering prices of productive services other than labor, our analysis must again be confined to the United States as a whole, because no adequate price data are available for California. It can be assumed that the level of California prices is somewhat higher because most productive services are imported from other parts of the country. More importantly, differences in weighting between California and the United States excluding California (p. 23) influence fluctuations of the over-all price index of productive services. It will be shown presently that prices of different groups of productive services behave differently during price fluctuations. But still, results obtained from an analysis of these individual groups for the United States as a whole are significant also for California.

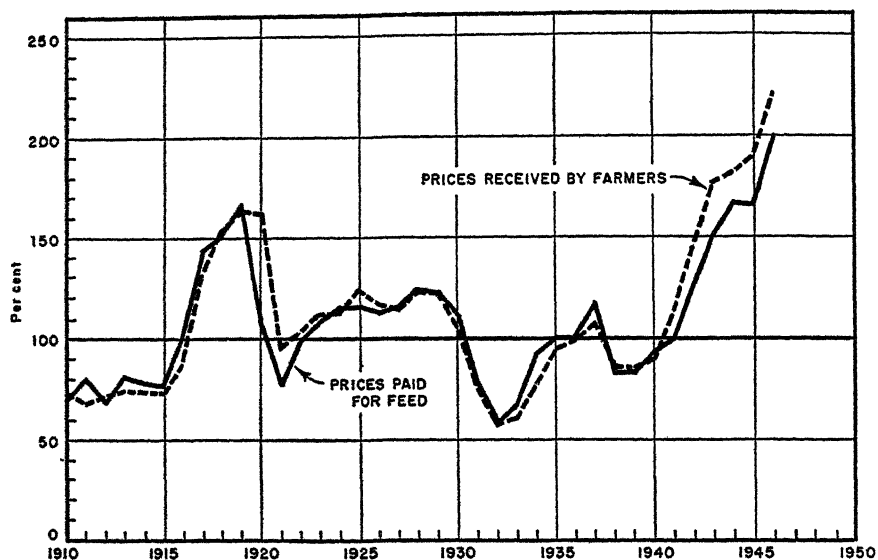
Prices paid by farmers for productive services other than labor fluctuate with a distinctly smaller amplitude than prices received (fig. 8, *B*). This tends to improve the economic position of farmers when prices rise and to worsen it when prices fall. The index of prices paid for productive services comprises several groups of prices, the movements of which warrant separate analysis for reasons already indicated.

There are, first, the fluctuations of feed prices (fig. 9, *A*). Feeds are largely farm-produced commodities. Fluctuations in feed prices, therefore, closely correspond to those of prices received. The relatively small differences between the two series are largely due to the fact that feed prices are more heavily weighted with grains. Feed prices, however, are obviously not the component of the general cost-price index (fig. 8, *B*) which is responsible for the smaller amplitude in the latter's fluctuations compared with those of prices received.

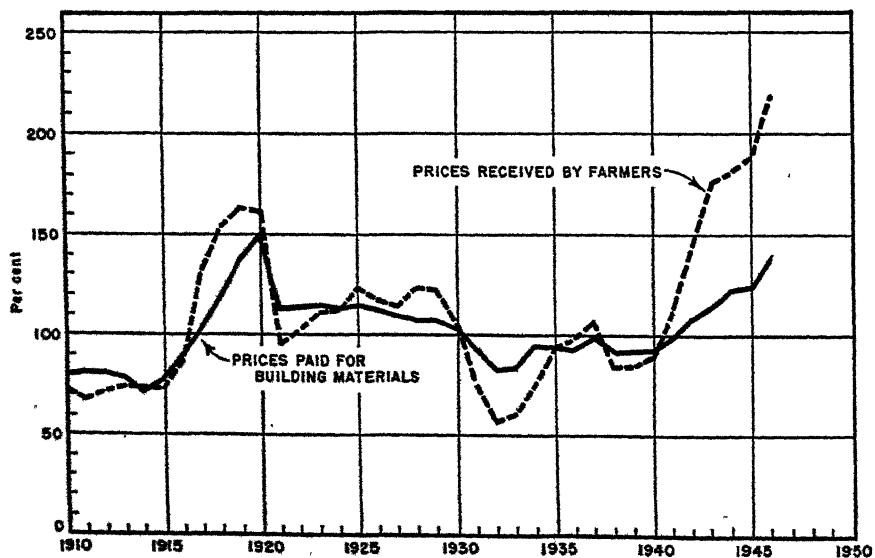
This situation is quite different with prices paid for farm machinery (fig.

FIG. 9. PRICES RECEIVED AND PRICES PAID

A, Prices Paid for Feed and Prices Received by Farmers

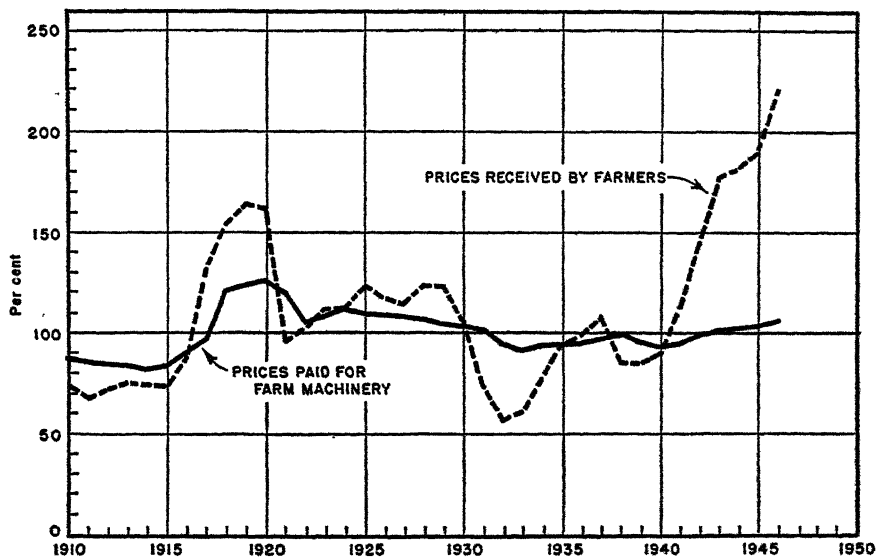


C, Prices Paid for Building and Fencing Materials (other than Houses) and Prices Received by Farmers

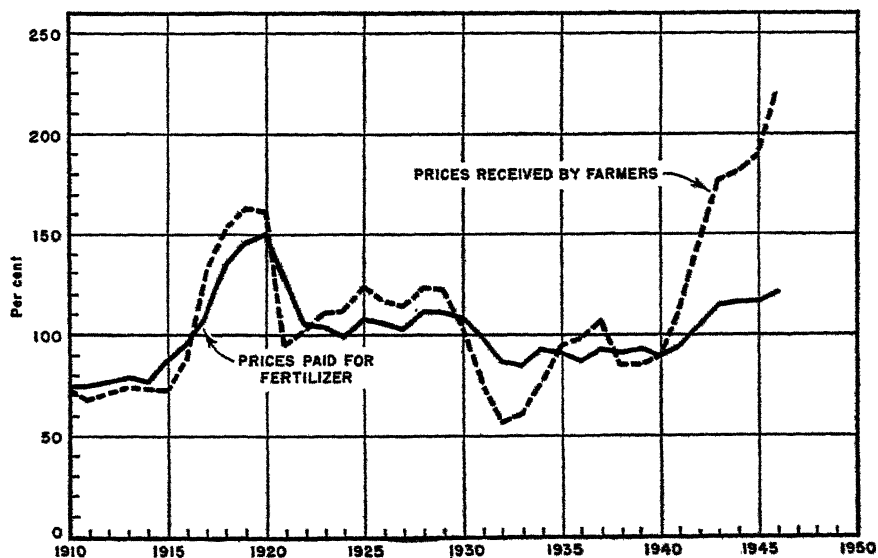


BY FARMERS, UNITED STATES (TREND = 100)

B, Prices Paid for Farm Machinery and Prices Received by Farmers



D, Prices Paid for Fertilizer and Prices Received by Farmers



9, *B*). These prices barely show fluctuations. The small fluctuations that are present correspond in direction to those of prices received, but with a lag of one year or more. It is less important that the trend was slightly upward ($b = +0.534$). This difference relative to the trend of prices received by farmers was probably more than offset by technological improvements of farm machinery. In other words, equal efficiency units of farm machinery have probably decreased in price relative to farm products.

The behavior of prices paid for farm machinery is also characteristic of prices paid for most other manufactured products. For example,¹³ prices paid for building and fencing materials (fig. 9, *C*) and for fertilizer (fig. 9, *D*) show much narrower fluctuations than do prices received.¹⁴ The degree of difference, however, is somewhat smaller than with farm-machinery prices.

The various, rather complex reasons for the rigidity of prices of manufactured products relative to prices received by farmers were treated in detail elsewhere (Ciriacy-Wantrup, 1939, 1940). Within the scope of this study it is sufficient to note that the difference of amplitude in the fluctuations of prices received and of prices paid for manufactured products is an important factor in changing the economic position of farmers in the course of general price fluctuations. It tends to improve their position during the upswing and to impair it during the downswing. This rigidity does not prevent stability of agricultural production—encouraged, as we have seen, by the flexibility of farm wage rates—because, during depressions, farmers can postpone buying machinery and fertilizer; they can use old equipment and deplete the soil.

Fluctuations in Taxes and Interest. Prices paid for labor, feed, and manufactured products are not the only items we have to consider. For California farmers as a whole, real estate taxes and mortgage interest are of considerable importance (p. 23). Taxes and interest show little resemblance to the fluctuations in gross income and production expenses, except a decrease after 1930 under the influence of foreclosures and of special public relief measures (fig. 10).¹⁵ The importance of the rigidity of charges for the economic position of farmers has often been emphasized, especially with respect to the adjustment period after World War I. This rigidity exists today also. However, there is an important difference in the level of these "fixed" charges between the situation after World War I and that prevailing now. Although taxes per acre have risen in recent years (fig. 10), the pre-1930 level has not been reached. On the other hand, net income per acre and land values have far surpassed their 1930 level. The interest burden has steadily fallen since 1930, partly because interest rates have fallen (see fig. 22, p. 56), and partly because farmers have used their increased income during World War II more

¹³ The two other groups of manufactured products in the general index of prices paid (fig. 7, *B*) are automobiles, trucks, and tractors, and materials and supplies. The former group price index is not available separately. The latter's behavior closely resembles that of building and fencing materials.

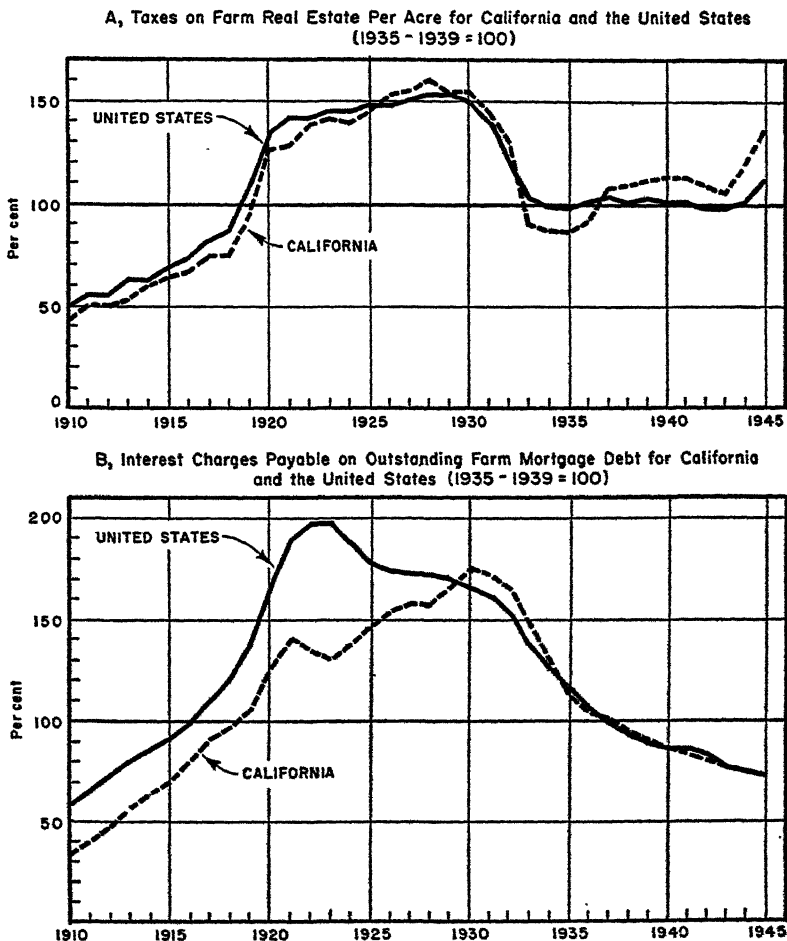
¹⁴ The trend of fertilizer prices is downward ($b = -0.410$) reflecting the rapid technological development in the chemical industries, particularly in the production of nitrogen. The trend of prices for building and fencing materials, like that for farm machinery, is upward ($b = +0.412$).

¹⁵ This lack of resemblance is so obvious that elimination of trend and comparison with prices received seemed unnecessary. Also, the data of fig. 10 are not strictly comparable with prices.

intelligently than during World War I: instead of purchasing land on credit, they have reduced their indebtedness.

Thus, although price fluctuations appear in a farmer's production expenses and charges as well as in his income, the changes are not all of the same ampli-

FIG. 10. FIXED CHARGES



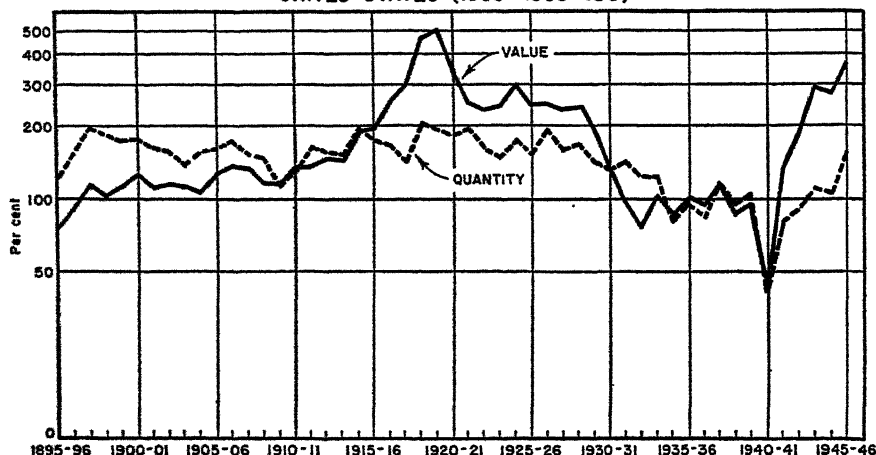
tude, nor do they all occur at the same time. These differences cause changes in his economic position in the course of a cycle. Hence the causes of price fluctuations are of fundamental interest to farmers. These causes will be explored in the following sections. It was shown in section 3 that production appears as a relatively unimportant factor in major fluctuations of cash farm income and agricultural prices, both in California and in the United States. As a next step in our analysis, therefore, we must turn to the demand side. First, we may ask how far changes in foreign demand may have been a factor.

5. FOREIGN DEMAND AS A FACTOR IN CHANGES OF CASH FARM INCOME

Summary. During the period surveyed, changes of foreign demand for agricultural exports were significant for changes of farm prices and cash farm income in the United States mainly because changes of foreign demand reinforced changes of domestic demand. Quantitatively, changes of foreign demand were less significant than changes of domestic demand.

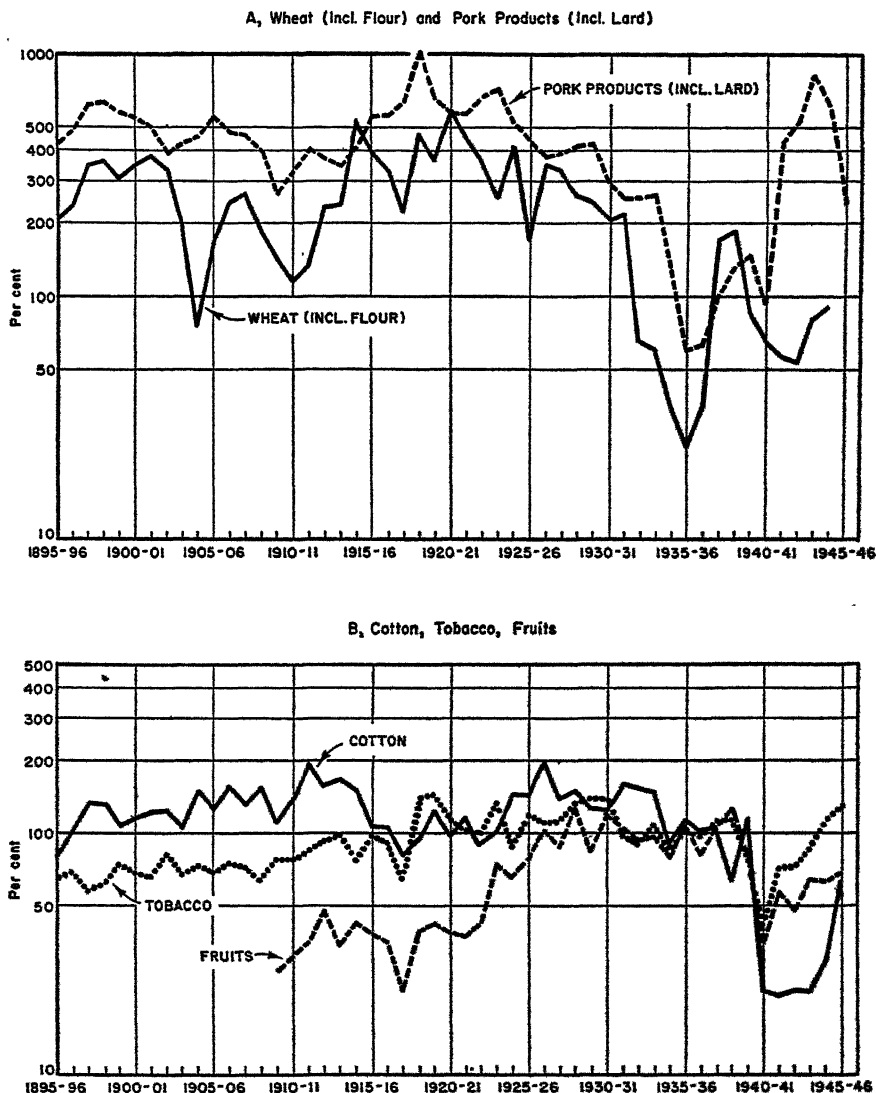
Value and Quantity of Total Agricultural Exports. Foreign-trade statistics make it possible to extend our period of analysis into the 1890's. Value and quantity of United States agricultural exports from 1895-96 to 1945-46 are shown in figure 11.

FIG. 11. VALUE AND QUANTITY OF DOMESTIC AGRICULTURAL EXPORTS, UNITED STATES (1935-1939=100)



From comparing the two series, the same conclusion comes to mind which was reached earlier from comparing cash farm income and quantities marketed: major fluctuations in the value of agricultural exports are more price than quantity phenomena. To be sure, quantity shows many oscillations; but these are relatively (compared with major fluctuations of value) short, and—at least, until the middle of the 1930's—they represent relatively small variations from a rather constant general level. This level, at around 150 (1935-1939=100), prevailed from 1895-96 to 1933-34. Changes of quantity in the same direction continued generally for not more than three years in succession and seldom reached 25 per cent of the level just mentioned. Value, on the other hand, shows changes in one direction of eight and ten years in succession; and variations (measured from the same level as that indicated for quantity) exceeded 200 per cent. Only between 1934-35 and 1940-41 did strong variations of quantity determine changes of value. The causes for these deviations of quantity were extreme harvest fluctuations in the United States

FIG. 12. QUANTITY OF DOMESTIC AGRICULTURAL EXPORTS BY MAJOR GROUPS OF COMMODITIES, UNITED STATES (1935-1939 = 100)



(largely effects of the drought in the middle of the 1930's) and the economic, political, and military difficulties of exporting to Europe in the beginning of World War II (in 1941, these difficulties were overcome by lend-lease and America's entry into the conflict).

Quantity of Agricultural Exports by Major Groups of Commodities. The quantity of agricultural exports warrants a more detailed examination by

major groups of commodities (fig. 12). These commodities represent about 90 per cent of United States agricultural exports. Two distinct patterns of behavior may be noted.

The first pattern (fig. 12, *A*) shows rather violent fluctuations. The major export commodities which show this pattern are wheat (including flour) and pork products (including lard). The latter may also be regarded as grain exports (mainly corn) in refined form. These groups of exports are greatly influenced by harvest fluctuations in the United States for two reasons: first, harvest fluctuations in grains are especially great; second, the export surplus is small in relation to total production. These groups of exports were also greatly affected by the two world wars: They are relatively cheap and concentrated sources of calories. They have, therefore, first priority for exports under conditions of shipping shortages during the war and of low foreign purchasing power during postwar readjustment.

The results of harvest fluctuations and of war obscure, but do not obliterate, a long-term downward tendency. This tendency, already noticeable during the fifteen years prior to World War I, was resumed in the beginning of the 1920's, and will, in all probability, reassert itself after the present postwar boom in exports has passed. The main cause for this long-term decline of exports was the expansion of the domestic demand in the United States, and the shifts in domestic production which took place under the stimulus of this expansion. This cause constitutes a permanent change in the structure of the American economy. Since the cause of the decrease in exports was an expansion of domestic demand, this decrease can scarcely be regarded as an important reason for declines of farm prices and of cash farm income. There is statistical confirmation for this opinion: The decrease in exports continued during the second half of the 1920's when farm prices and cash farm income were rising; again, the decrease was not especially great around 1920 and 1929 when major breaks in prices and cash farm income occurred.

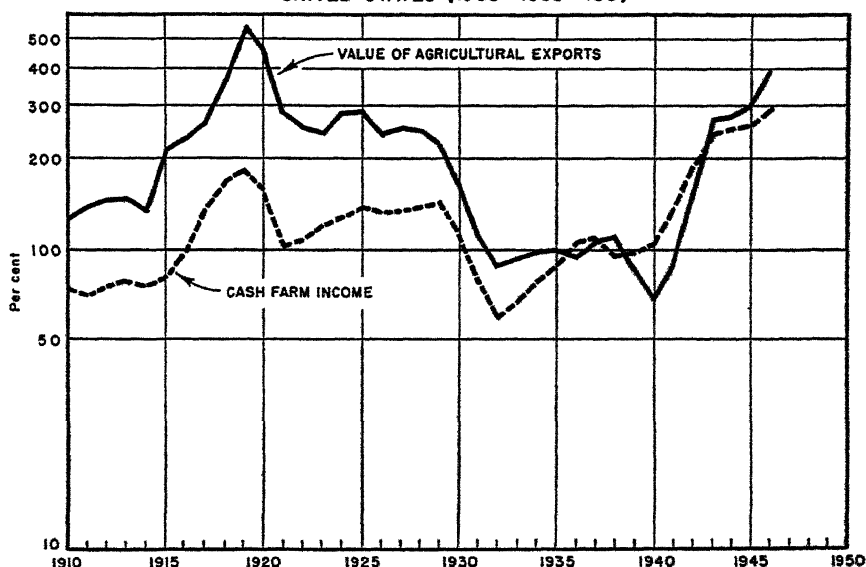
The second pattern does not show a long-term decrease (fig. 12, *B*). On the contrary, until the first half of the 1930's, the trend was upward. From then on, until the outbreak of World War II in 1939, exports were well maintained. The effects of the drought in 1934 and 1936 and of the two world wars are smaller than in the first pattern. Cotton, tobacco, and fruits exhibit this pattern. These are products in which the United States had large exportable surpluses in spite of harvest fluctuations and the expansion of the domestic market. This will probably apply also to the future if government policies, which artificially reduced exportable quantities and deliberately priced the American product out of world markets, are discontinued. These policies were particularly important for cotton. For the three commodities as a whole, however, there is no indication that changes of exported quantities were responsible for the major fluctuations of total value of agricultural exports observed above (fig. 11).

Value of Agricultural Exports and Cash Farm Income. Turning now to major fluctuations in the value (in contrast to quantity) of agricultural exports, a positive correlation with fluctuations of cash farm income exists (fig. 13). This was to be expected because, as already mentioned, fluctuations of both series are dominated by fluctuations of farm prices rather than quan-

tities. From this correlation far-reaching conclusions for the significance of agricultural exports for United States farmers have been drawn. The most clearly formulated example concludes a recent study of the U. S. Department of Commerce (1946) and may be quoted in full:

However, a change of one dollar in agricultural exports appears to effect an average change of approximately \$1.60 in cash farm income, on the basis of the long-term relationships described above. Part of the rise in income is attributable to the higher prices received for the reduced quantity of products sold in the domestic market. Since the domestic demand for most farm products is rather inelastic, a reduction in the available supply, such as that which takes place when exports expand, ordinarily results in a more than proportional rise in price. Similarly, any major decline in exports is likewise important.

FIG. 13. VALUE OF AGRICULTURAL EXPORTS AND TOTAL CASH FARM INCOME, UNITED STATES (1935 - 1939 = 100)



How far can this conclusion be accepted? In spite of the oversimplification involved, one may grant that the domestic demand for agricultural products as a whole is "rather inelastic" or, more precisely, that its price elasticity is smaller than unity over the relevant range. Likewise, within a short period—a year, for example—the total supply for domestic use and for export may be regarded as a fixed quantity or, at least, of small price elasticity. Under these assumptions any increase of demand, foreign or domestic, must lead to a considerable price increase in the domestic market.¹⁸ Such effects should not be credited especially to an increase of foreign demand.

¹⁸ The phrase "more than proportional rise in price" (used in the quotation above) is avoided because we are dealing here with increases of demand in the sense of upward shifts of the demand curve rather than movements along the "old" curve. It will be shown later that such shifts under the influence of changes of nonagricultural income and business activity are the essential problem in economic fluctuations. It cannot be assumed *per se* that the smaller the elasticity of the demand function, the greater the price rise from a given upward shift of the demand function.

Whether an increase of domestic prices (caused by an increase of foreign or domestic demand) leads to an increase of cash farm income depends on the price levels in the domestic and foreign markets and (if these levels are not identical) on the proportion of exports to total marketings. If the two price levels are identical, an increase in domestic prices must lead to an increase of cash farm income. As a special case, this situation may be assumed to have prevailed for agricultural export commodities during the period under consideration.

Nevertheless, there are two reasons why the proportion of the value of agricultural exports to cash farm income is significant for an analysis of the importance of foreign markets for United States farm products.

It was just indicated that, in principle and under the conditions specified, changes of domestic demand for agricultural products have the same importance for farm prices and incomes as changes of foreign demand. In actuality, effects depend on the magnitude of the changes and on the proportion of agricultural exports to total farm marketings. Changes of equal relative magnitude have much greater effects if they occur in domestic demand rather than in foreign demand. It is shown by the following data, giving the ratio of the value of domestic agricultural exports to total cash income from farm marketings in the United States,¹⁷ that domestic demand has been of increasingly greater importance (in this sense).

Year	Per cent	Year	Per cent	Year	Per cent
1910.....	16.4	1922 . . .	21.9	1934.....	11.6
1911.....	18.5	1923.....	19.0	1935.....	10.5
1912.....	18.0	1924.....	20.6	1936.....	8.5
1913.....	17.9	1925.....	19.4	1937.....	9.0
1914.....	21.4	1926.....	17.2	1938.....	10.8
1915.....	25.1	1927.....	17.5	1939.....	8.3
1916.....	22.6	1928.....	16.8	1940.....	6.2
1917.....	18.4	1929.....	15.0	1941.....	6.0
1918.....	20.4	1930.....	13.3	1942.....	7.7
1919.....	28.0	1931.....	12.9	1943.....	10.7
1920.....	27.3	1932.....	13.9	1944.....	10.4
1921.....	25.9	1933.....	13.1	1945.....	10.9

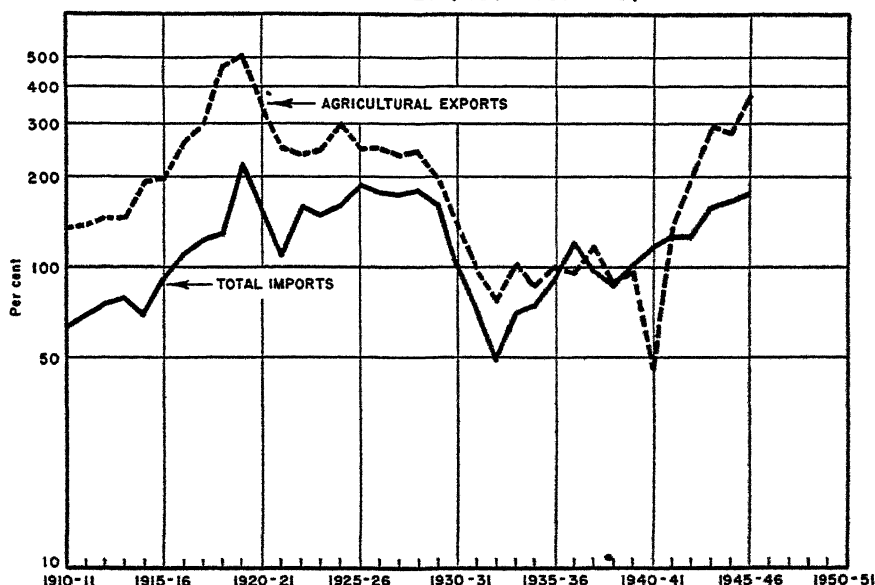
These conclusions apply to American agriculture as a whole. For certain products—for example, cotton—the importance of foreign demand approaches that of domestic demand.

The proportion of agricultural exports to cash farm income has analytical significance also for a negative reason: explaining the regression coefficient of \$1.60 mentioned in the quotation above. If relatively small values (of agricultural exports) are correlated with relatively large ones (cash farm income) and the latter are regarded as a dependent variable, the regression coefficients must be larger than one dollar merely as a matter of statistical “mechanics.” The conclusion “a change of one dollar in agricultural exports appears to effect an average change of approximately \$1.60 in cash farm income” does not necessarily follow. This, at least, is true if the word “effect” is interpreted as indicating a cause-effect relation—as in the quotation.

¹⁷ Although the proportion is shown in terms of value, it may also be interpreted in terms of quantity because the price factor is approximately the same for both series.

Besides the proportion of agricultural exports to cash farm income just considered, another factor points to the greater importance of domestic demand in interpreting the correlation shown in figure 13. Foreign demand for American agricultural products is determined by the availability of dollar exchange in foreign countries. In peacetime—that is, excluding war-related gifts called by whatever name (war loans, lend-lease, U.N.R.R.A. contributions, relief to liberated and occupied countries, reconstruction loans)—such availability is dependent upon American imports of foreign products and services. This dependence during the interwar period is shown in figure 14.

FIG. 14. VALUE OF AGRICULTURAL EXPORTS AND OF TOTAL IMPORTS, UNITED STATES (1935 = 100)



The correlation would probably be even greater if imports of services could be considered. Unfortunately, reliable statistics on this point are not available.

United States demand for imports is determined by the same forces that determine domestic demand for United States agricultural products; these forces will be discussed in detail in following sections. During the interwar period, foreign and domestic demands for agricultural products were closely interrelated for this reason. During the two war periods, exports of United States agricultural products did not depend on United States imports. Still, foreign demand for agricultural products was related to domestic demand, because both were determined by the same international factor, namely, war needs.

On the basis of the theoretical considerations in the preceding paragraphs and of the earlier discussion of the behavior of export quantities (figs. 11 and 12), we may attempt to interpret the correlation shown between agricultural

exports and cash farm income (fig. 13). During the period surveyed, changes of foreign demand for agricultural exports were significant for changes of farm prices and cash farm income in the United States mainly because changes of foreign demand reinforced changes of domestic demand. During the last thirty-five years, agricultural exports have not been a stabilizing factor for the American agricultural economy—as is widely believed. Quantitatively, changes in agricultural exports were less important than changes of domestic demand. During the interwar period, changes of agricultural exports were largely the result of domestic changes of demand and supply. During the two war periods, changes of agricultural exports were the result of the same factors that caused changes of domestic demand. In general, analysis of agricultural exports as a factor affecting changes of cash farm income points to the importance of changes in domestic demand. This problem will be considered in the next section.

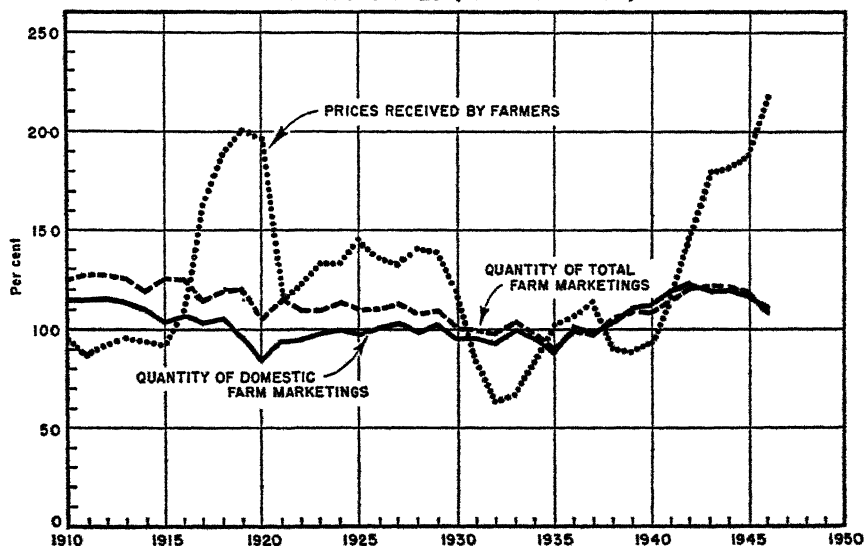
6. DOMESTIC DEMAND AS A FACTOR IN CHANGES OF CASH FARM INCOME

Summary. There is no indication that changes in number, composition, or diet of the nonagricultural population were responsible for major fluctuations in domestic cash farm income and agricultural prices. Changes in aggregate income of the nonagricultural population, especially of industrial workers, were highly correlated with fluctuations in agricultural prices and cash farm income. Other income factors—namely, changes in the proportion of income spent for agricultural products and changes in liquid funds—were either related to effects of changes in aggregate income or were of relatively small importance for the demand for agricultural products. But we cannot definitely conclude that fluctuations in aggregate income of nonagricultural population (especially of industrial workers) was the determining factor for fluctuations of agricultural prices. Income and price fluctuations may both be related to a common third factor or group of factors.

Aspects of Domestic Demand. Two aspects of domestic demand for farm products may be considered: the physical and the economic. The former includes number, composition, and diet of the nonagricultural population. The latter includes expenditures for farm products by final consumers and purchases by industry, processors, and traders. The various factors are interrelated. They may be treated separately, because they differ greatly in their importance for fluctuations of cash farm income and agricultural prices. Changes in number and composition of the nonagricultural population are small per year or even per decade and, except for the effects of economic fluctuations, are rather constant in direction. In diets, in consumer expenditures, and in purchases of nonagricultural industries, on the other hand, great changes may take place within a short period of time—a few months, for example—and the direction of these changes is rather variable.

Number and Composition of the Nonagricultural Population. Let us consider, first, the physical aspects of the domestic demand for farm products. In figure 15, quantities of total and of domestic farm marketings per person of the nonfarm population are compared with agricultural prices. Total quantities marketed per person of the nonfarm population decreased steadily until the middle of the 1930's. Domestically marketed quantities per person were maintained only because exports per person decreased. The decrease of exports, as we know, was largely confined to wheat and corn—the latter in the

FIG. 15. QUANTITY OF TOTAL AND OF DOMESTIC FARM MARKETINGS PER PERSON OF THE NON-FARM POPULATION AND PRICES RECEIVED BY FARMERS, UNITED STATES (1935-1939=100)



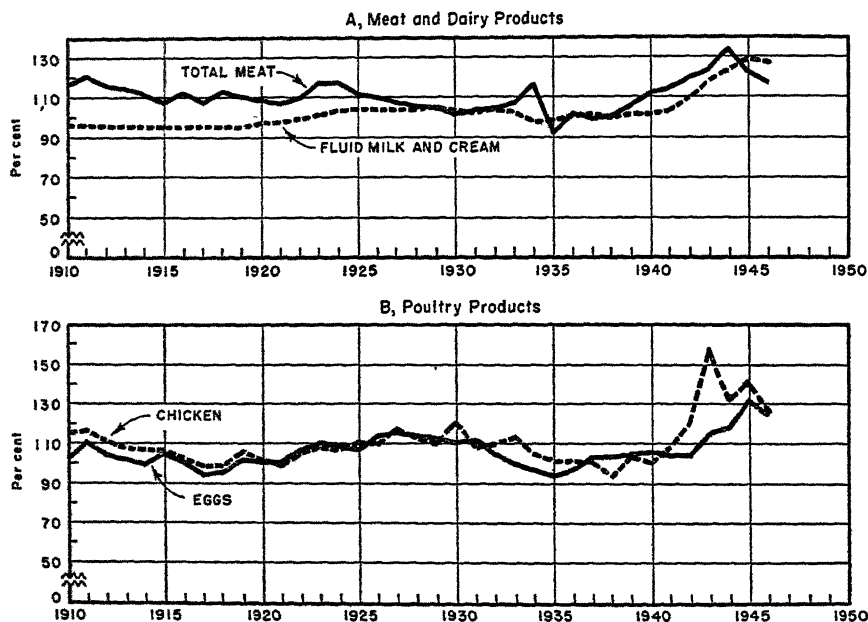
form of pork products (section 5). Since the middle of the 1930's, supplies per person increased gradually with rising prices.

There is no indication that changes in prices were caused by changes in the supplies per person. On the contrary, some of the more important changes of quantities—for example, the decrease after 1929 and the increase since the middle of the 1930's—correspond in direction with changes of prices. This picture is not altered by taking into account changes in the composition of the nonagricultural population: during the period under review, especially during the "great depression" after 1929, the proportion of full consumers in the nonagricultural population increased.

Diet. The most significant aspects of changes of diets are changes in the consumption per person of animal products. Such changes alter the physical absorptive capacity per person for crops because of the great losses in feeding. These effects are taken into account in our quantity indices (fig. 15), only so far as grain and other crops are fed on the farm without market transactions. Some animal production is based on purchased feed. It is, therefore, necessary to review briefly changes in the consumption of animal products.

Figure 16 shows domestic consumption of total meat, fluid milk and cream, eggs, and chicken per person.¹⁸ It is evident that changes in the domestic consumption of animal products per person were in the same direction as changes in total domestic farm marketings per person (fig. 15);¹⁹ the amplitude of the former changes, especially the increase after the middle of the 1930's, was certainly not less (but possibly greater) than the amplitude of the latter changes. Variations in supplies per person for human consumption can, there-

FIG. 16. DOMESTIC CONSUMPTION PER PERSON: TOTAL MEAT, FLUID MILK AND CREAM, EGGS, AND CHICKEN, UNITED STATES (1935-1939 = 100)



fore, be no greater (but possibly may be less) than the small variations in domestic farm marketing per person shown in figure 15. In so far as shifts of consumption between crop and animal products in human consumption are not induced by income changes, they are a stabilizing influence in economic fluctuations. Such shifts act as a "buffer" in fluctuations of crop production. On the other hand, in so far as these shifts are induced by income changes, they

¹⁸ For dairy and poultry products, consumption per person could only be shown for the civilian population. The increase in the consumption of animal products per person since 1941 would be considerably greater if military consumption had been included. Because of greatly increased military consumption of butter, cheese, and condensed and dried milk after 1941, and because of the strict rationing of these products for civilian consumption, total milk equivalent does not show the same strong increase as the other series. Civilian consumption of fluid milk and cream during this period is a better indication of actual changes in over-all (including military) consumption of dairy products than total milk equivalent.

¹⁹ Total meat consumption shows the same strong increase after the middle of the 1930's as domestic consumption per person of dairy and poultry products. On the other hand, during 1933 and 1934, there is a sudden reversal of the downward trend when the other two series decreased further. This was caused by abnormally heavy slaughtering on account of the drought, accentuated by government programs,

tend to accentuate the income effects. During an upswing of income the increase in demand is especially great for animal products. This accentuates the increase in the over-all (including feed) demand for crops. During a downswing of income the opposite development takes place.

By some students the decrease of physical absorptive capacity for crops through replacement of farm draft animals by tractors has been regarded as a potent factor in creating agricultural "surpluses." It may be well, therefore, to point out that the great decrease of farm draft animals during our period of analysis and the corresponding release of crop production for human consumption is, for the purpose of this discussion, fully taken into account in our quantity indices of farm marketings (fig. 15): feed for farm draft animals is overwhelmingly home-produced.

Three Income Factors. Turning now to economic changes of domestic demand, three main factors are connected with income of final consumers. These factors are, first, changes of aggregate income; second, changes in the proportion of consumer income expended for agricultural products (the preceding factor being held constant); third, liquid funds in the hands of final consumers which may be increased or decreased to cushion income changes.

Among the three income factors, changes of aggregate income are by far the most important. Nevertheless, before these changes are discussed in detail, the other two factors deserve a general consideration.

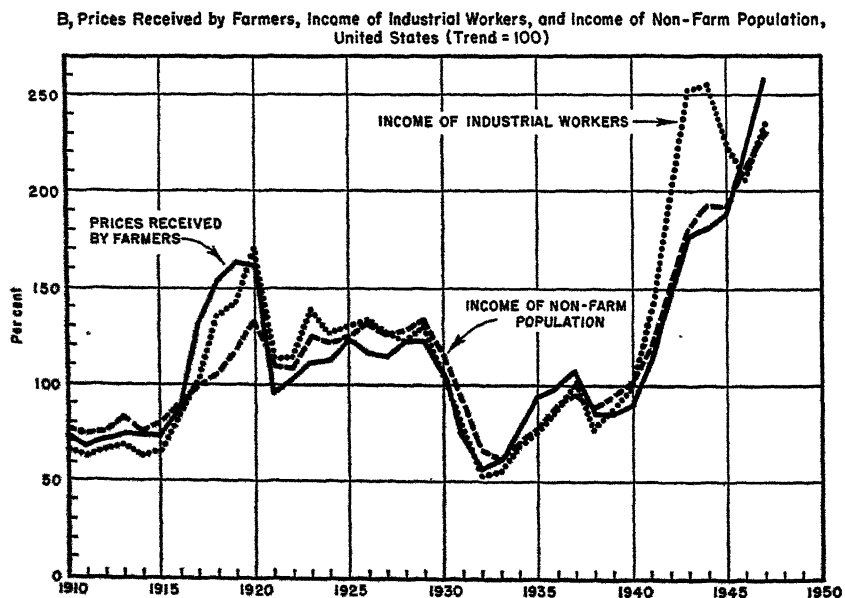
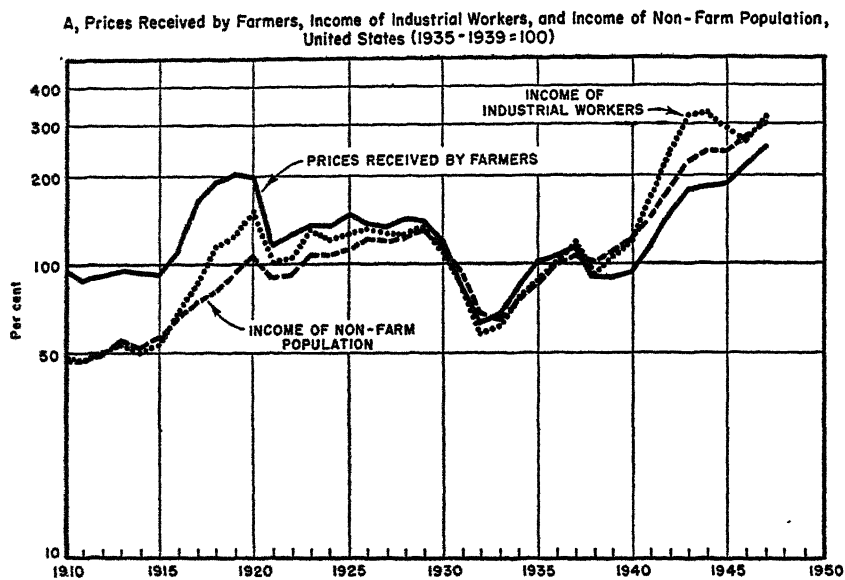
Income Distribution. Changes in the proportion of income spent for agricultural products may be caused by changes in income distribution.²⁰ Studies of consumption show rather conclusively that the lower-income brackets have a higher income elasticity of demand for agricultural products (especially food) than the higher-income brackets (U. S. National Resources Committee, 1939—see part 2, sec. 6, for references to previous pertinent studies; U. S. Bureau of Home Economics, 1941*a*, 1941*b*, 1942; U. S. Bureau of Labor Statistics, 1941). However, changes in income distribution with constant aggregate income proceed rather gradually if revolutionary changes of economic institutions are excluded.²¹ Changes of income distribution caused by changes of aggregate income in the course of economic fluctuations are likewise small, but probably accentuate the effects of changes in aggregate income upon cash farm income and agricultural prices.²² As we shall see later, changes in aggre-

²⁰ Changes in the proportion of income spent for agricultural products may also be caused by changes of prices for reasons other than income changes. Price elasticity of the demand for agricultural products is frequently different from that of nonagricultural products. Likewise, governmental controls applied differently in degree to agricultural products and to products competing with agricultural products for the consumer's dollar (for example, durable consumer goods) may affect this proportion. We are not concerned here with these nonincome factors.

²¹ Even great changes of economic institutions, provided they proceed gradually, have apparently small influence upon the income distribution. In Great Britain, for example, income distribution changed little between the Napoleonic Wars and the first world war in spite of the great impact of the industrial revolution.

²² By readers who are familiar with the concept of the "consumption function" it may be noted that the accentuating effect referred to in this paragraph does not necessarily contradict the dampening effects resulting from shifts of the consumption function during economic fluctuations and from changes of elasticity at different income levels (without shifts). However, nature and extent of shifts and changes of elasticity of the consumption function need more investigation before definite statements about them can be made. We shall return to these problems in the following section.

FIG. 17. PRICES RECEIVED BY FARMERS AND NONAGRICULTURAL INCOME, UNITED STATES



gate income affect industrial workers more than the nonagricultural population as a whole (fig. 17). Wage earners, particularly unskilled and semiskilled industrial workers, comprise the lower-income brackets of the nonagricultural population.²³

Liquid Funds. Changes in the accumulation of liquid funds influence the demand for agricultural products, but less than the demand for most non-agricultural products. Recent investigations have shown that accumulations of liquid funds are concentrated in the higher-income groups, and that the inequality in liquid-funds distribution is even greater than the inequality in income distribution (U. S. Bureau of Agricultural Economics, 1945-46). Since income elasticity of demand for agricultural products in the higher-income groups is small, it can be expected that "liquid-funds elasticity" of demand for agricultural products is even smaller. It may be well to mention that we are interested at this point in the "direct" relations between changes of liquid funds in the hands of final consumers and changes of demand for agricultural products. Changes in liquid funds (in the hands of final consumers and of others) may have significant relations with activity in nonagricultural industries and, through income, with demand for agricultural products. These "indirect" relations will be considered in the next section.

Aggregate Income. After this general appraisal of the two less important income factors, we may focus on changes of aggregate income. Since we know that, in agriculture, economic fluctuations are largely price and not quantity phenomena, we may compare prices received by farmers with the aggregate income of the nonagricultural population and of industrial workers (fig. 17). Because of relations already observed (fig. 5), this comparison applies also to cash farm income.

In contrast to prices received by farmers, the two income series show the same strong upward trend (fig. 17, A). Major fluctuations, however, are similar for all three series. This becomes especially clear after differences in trend are eliminated (fig. 17, B). Income of industrial workers shows the strongest fluctuations. Income of the nonagricultural population contains important items (income from salaries, rents, interest, professional services) which are less affected by changes in business activity than industrial payrolls.

About one third of the income of the nonagricultural population consists of income of industrial workers. If the latter were subtracted, income of the rest of the nonfarm population would show even greater stability. During the first world war and during the interwar period, fluctuations of farm prices corresponded more closely to fluctuations of industrial payrolls than to those of nonagricultural income as a whole. The same would probably have occurred during the second world war if farm prices had not been kept under government control. The effect of these controls requires some explanation.

In part, government controls merely obscured the influence of industrial payrolls upon farm prices: official price statistics do not sufficiently take

²³ Unfortunately, it is statistically not possible to study the income of unskilled and semi-skilled industrial workers separately from that of skilled workers. The income of industrial workers shown in fig. 17 is largely earned by skilled workers (factory, mining, Class I railway employees). If this statistical difficulty could be overcome, differences in amplitude of income fluctuations would probably appear more pronounced.

account of black markets, upgrading, quality deterioration, and nonavailability of certain groups and grades of commodities. In part, however, government controls were effective in cushioning the full impact of the great increase of industrial payrolls upon farm prices. First, through various forms of subsidies, producers' and processors' incomes were increased without increasing farm and retail prices. Second, through rationing and price ceilings, the effect of increased payrolls upon both prices and producers' incomes was prevented, or—more correctly—delayed. The effect appeared when farm prices rose faster than industrial payrolls after government controls were released in the middle of 1946 (fig. 17, *B*). It is impossible to measure the influence of the foregoing factors accurately. All of them were especially important when most of the discrepancy between industrial payrolls and farm prices occurred, between 1943 and the first half of 1946.

The correspondence between fluctuations of prices received by farmers and our two indicators of nonagricultural income is rather close. In terms of relative deviations from trend, correlation coefficients between prices received by farmers, on one side, and income of industrial workers and of the nonagricultural population, on the other side, are 0.91 and 0.92 respectively for the period 1910–1946. The correspondence would be even closer if the effect of the other three income factors and of government controls could be measured more accurately. It is safe, then, to conclude that fluctuations of prices received by farmers and of cash farm income are mainly caused by fluctuations of nonagricultural income?

●

Investigation of nonagricultural income has led us very close to an explanation of fluctuations of farm prices, but there are two reasons why an affirmative answer to the last question needs some qualifications:

First, it was indicated above that many farm products are not bought with income of final consumers. Many agricultural products are industrial raw materials—for example, cotton, other fibers, hides, tobacco, and a considerable part of the grain and soybean crop. Industrial raw materials of agricultural origin are bought by business funds of industry and trade. Likewise, many agricultural products, other than industrial raw materials, are bought by funds of processors rather than final consumers. For storable products, changes of inventories in the hands of the trade are important. For products for which a market for futures exists, speculation may at times play a significant role. The period of manufacture and of processing, variations of inventories, and speculation in the market for futures may modify the effects of changes of final consumer's income upon agricultural prices and cash farm income. The question arises, therefore, whether business activity in these economic sectors is also determined by income of final (nonagricultural) consumers, or whether both are related to a third factor or group of factors. The correlations shown in figure 17 do not answer this question.

Second, farmers and policy makers cannot be satisfied with having fluctuations of agricultural prices explained by fluctuations of nonagricultural income. They will naturally ask: What causes fluctuations of nonagricultural income? An answer to this question will also provide answers to the preceding one. An attempt will be made to explore this problem in the next section.

7. FACTORS AFFECTING CHANGES OF NONAGRICULTURAL INCOME

Summary. Theoretical consideration of the processes of income formation in the modern economy point to an unbalance between saving and investment as the crucial factor affecting changes of nonagricultural income. An analysis of changes of saving and investment shows that changes of saving (and consumption) are income-induced and income stabilizing. Changes of investment, on the other hand, are to a large extent independent of income changes, are relatively more violent than changes in consumption, and are accentuated by secondary effects upon consumption and investment. Changes of investment, therefore, appear as a major cause of changes of nonagricultural income. Prices received by farmers are affected by changes of investment not only through nonagricultural income, but also directly through purchases of agricultural raw materials and changes of inventories. Changes of investment have also important effects upon total imports and through them upon exports of agricultural products. Changes of investment explain changes of domestic demand for agricultural products and also changes of foreign demand.

Processes of Income Formation. Changes of nonagricultural income and the causes of such changes belong to the most important aspects of the modern economy. An understanding of these aspects by farmers is essential for making proper decisions in their own business and, even more so, for taking part constructively in the formation of public economic policies. Before interpreting the available statistical data, it is necessary, therefore, to consider in general terms the processes of income formation in the modern economy.

To insure stability (see explanation in the next paragraph) over time of a given aggregate money income, of a given price level, and of a given employment level at given productivity of the working force, as much income must be returned to the income stream in each turnover period—for example, every three or four months, according to the income velocity of money—as was received in the previous period. Strictly speaking, employment and productivity involve three independent variables: first, the working force; second, the hours of work per man; and, third, the product per man-hour. For our purposes the latter two variables are included in productivity. For forecasting and for a definition of “full” employment, however, a clear differentiation between these three variables is necessary.

The foregoing statement requires an important qualification of what is meant by the term “stability”: in a growing economy, like that of the United States, the working force is increasing and so is its productivity, in spite of decreasing hours of work per person. Under these conditions, stability of money income per person, of the price level, and of employment (in the sense that involuntary unemployment remains unchanged) is insured only if increasing amounts of income are returned to the income stream in each turn-

over period. In other words, stability of income in terms of a rising trend (because of increases of the working force and its productivity) is under discussion. This trend is illustrated in figure 17, A. We could also express income in terms of wage units—as was done elegantly by Keynes (1936). However, such treatment of the growth factor obscures rather than elucidates some vital problems of economic stability.

A portion of income is collected as taxes and usually returned to the income stream through government expenditures.²⁴ By far the larger portion of the remaining (after taxes) income is returned to the income stream in the form of consumption. A relatively small portion is saved.²⁵ The crucial problem is what happens to these savings. If they are used by private industry or by the government and returned to the income stream as investment (to be explained presently), no diminution in this stream takes place. But if savings accumulate as idle balances in the hands of individuals, industry, banks, or government, the income stream is decreased. The decrease of income exercises pressure upon prices. If prices are flexible, a new equilibrium between the demand for idle balances and the demand for goods (consumption and investment) may be reached at a lower price level. At best, such an adjustment is slow and painful. If prices are not flexible—that is, if the economy adjusts to decreasing incomes largely through decreases of production rather than prices—a vicious circle is set in motion until saving is decreased in order to maintain consumption, or until investment increases.

Meaning of Investment. The term investment is used with many meanings in scientific as well as popular literature. With respect to income formation, investment means the expenditure of liquid funds (made available by current and accumulated saving and by the creation of “new” funds through the banking system)²⁶ for increases of inventories and for the production of durable goods. These goods are either producer durable goods—for example, factories, industrial equipment, irrigation systems, railroads; or government durable goods—for example, roads, dams, offices, and armaments; or consumer durable goods—for example, houses, automobiles, radios, and appliances.

Differentiation between investment in capital goods—that is, producer and government durable goods—and in consumer durable goods is desirable from the standpoint of income formation. It may be even argued that consumer durable goods are better excluded from a definition of investment. However,

²⁴ Tax-collected funds may be “saved” (see next footnote) by governments. Such action belongs logically in the field of fiscal policy to influence the income stream, and will be considered in section 9.

²⁵ In scientific usage, “saving” means the use of income for increasing money hoards, saving and checking accounts, for debt repayments, for payments of insurance premiums, and for the purchase of readily marketable securities. New debts and decrease of hoards, accounts, and security holdings are dissaving. In speaking about the economy as a whole, “net” saving (after taking account of dissaving) or “net” dissaving (after taking account of saving) is usually meant.

In this study saving is used in the “*ex ante*” (or “intended”) sense; this means that saving in one turnover period is compared with investment (see the definition in the text) in the succeeding period. It is believed that this use of the term facilitates an understanding of changes in the income stream from period to period. By some authors, saving is used in the “*ex post*” (or “statistical”) sense; this means that saving and investment are considered for the same turnover period. In this case saving is equal to investment by definition.

²⁶ The creation of “new” funds is mentioned separately from saving because they are not withdrawn from the income stream. Some authors include such funds in saving. Under the “*ex post*” definition of saving (see preceding footnote) this becomes necessary.

in a modern economy with increasing aggregate and individual importance of consumer durable goods, a clear differentiation between capital goods and consumer durable goods is practically and theoretically difficult. In many respects, consumer durable goods have characteristics similar to those of capital goods. They are purchased by accumulated rather than current saving, or on credit. Their purchase can be postponed over a considerable time. Processes and periods of production of consumer durable goods are similar to those of capital goods. Examples are houses and factories, automobiles and trucks, domestic appliances, and industrial equipment. On the other hand, investment in consumer durable goods is generally income-induced; whereas investment in capital goods is largely independent of income. Furthermore, fluctuations in durable consumer-goods industries—although more violent than fluctuations in the nondurable consumer-goods industries—are less violent than fluctuations in capital-goods industries; and, sometimes, the former lag behind the latter. For reasons which will become clear presently, independence from income is a major consideration for defining investment. On the basis of this consideration, and certain statistical ones explained later, consumer durable goods were excluded from our composite indicator of investment (p. 50).

The term may be used as gross investment if replacement and maintenance of existing durable goods are included, or as net investment if these items are excluded. In discussing economic fluctuations it is sometimes convenient to use investment in the former sense. In this case saving (and income) includes reserves for replacement and maintenance.

Equilibrium between Investment and Saving. We may say, then, that the essential problem of stabilizing the income stream from period to period is that the rate of investment be in equilibrium (balance) with the rate of saving. The term "is in equilibrium (balance)" instead of "is equal" is chosen because, as already emphasized, in a growing economy the investment rate must be larger than the saving rate in order to stabilize income per person and the levels of prices and employment. How much larger the investment rate must be depends on the rate of increase in the working force and its productivity.²⁷ Investment in producer durable goods itself is usually (but not necessarily) an important factor in increasing productivity.

If the investment rate is less than this equilibrium rate, an economic depression results: the income stream contracts, prices decrease, and involuntary unemployment increases. If prices are not flexible (see p. 44), and if no new external investment stimuli (see the next section) occur, contraction may continue until a certain "base level" of income and employment is reached. At this level, the unbalance between the rates of saving and of investment has disappeared through decrease of saving in order to maintain consumption.

If the rate of investment is greater than the equilibrium rate (because accumulated savings are drawn upon or funds newly created by the banking system are used), the income stream is increased. This increase leads to an increase in the stream of goods produced as long as there are unemployed resources and no serious bottlenecks. After full employment of resources is reached, any further excess of investment over its equilibrium rate leads to

²⁷ Habits of payments (income velocity of money) assumed to remain unchanged.

income and price inflation without increase in the real social product. Sooner or later a collapse of investment occurs because of stresses in the monetary system, distortions in the price structure, and because external investment stimuli have weakened.

The question may now be asked: Why does an unbalance between the rate of saving and of investment occur? First, we may consider the behavior of saving and, second, that of investment. For brevity's sake we shall henceforth speak of income, saving, consumption, and investment, although (if not otherwise indicated) rates per turnover period are meant.

Significance of Saving. In recent economic literature a great deal of attention is given to saving. It is frequently implied that saving, far from being a virtue, is actually an important contributing cause to economic fluctuations. Many economists believe that a decrease of saving in favor of consumption is the most important prerequisite for future stability of income in the American economy. How far can this view be accepted?

The proportion of income after taxes that is saved or consumed changes little spontaneously—that is, without changes of income (excluding the effects of forcibly deferred consumption during a war).

The influence of the rate of interest upon this proportion is frequently overestimated. This influence is small partly because other factors (habit, considerations of prestige and security) are more powerful than variations of interest rates, and partly because the reaction of different groups of savers to variations of interest rates is different in sign. Some groups save more when interest rates increase because the inducement to accumulate wealth for its own sake is greater or because (if savers are interest recipients) possibilities for such accumulations are greater. Other groups save less when interest rates increase because they save for a minimum future income; annuities and income-yielding assets become cheaper when interest rates increase. Likewise, the possibilities to save are curtailed through an increase of interest rates for those who are in debt.

In secular perspective, greater urbanization of the American economy tends to decrease the proportion of income saved: the propensity to save of the rural population is greater than that of the urban population of equal income status. (However, age distribution and size of family may be, at least partly, the cause of this difference rather than occupation and residence.) A greater proportion of older people in the population probably tends in the same direction. So does a greater equality in the income distribution. More complex changes are related to changes of taxation.

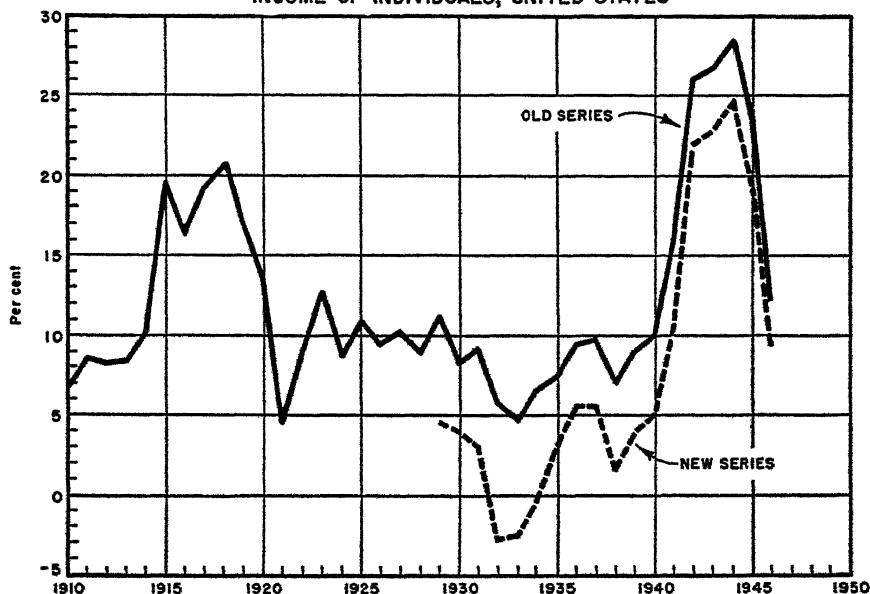
It was mentioned previously that the proportion of income (after taxes) saved changes under the influence of income changes. Such changes of saving as a function of income are considerable during cyclical (in contrast to secular)²² changes of income (fig. 18).²³ With increasing income, during eco-

²² Much of the confusion in present economic theory about the constancy of the "consumption function" is caused through lack of clarity in differentiating between secular and cyclical changes.

²³ The new series for income and consumption is not available before 1929. For this reason the old series is also presented. In the new series, savings are considerably smaller than previously estimated. However, cyclical fluctuations of the ratio of saving to income are as great (if not greater) in the new series as in the old. These fluctuations, rather than the magnitude of the ratio, are relevant for the argument presented in the text.

economic fluctuations, the proportion of income saved tends to increase, and with decreasing income to decrease. These effects are especially great in the beginning of income changes, before new consumption patterns based on the new income level are established. During the two war periods, saving was increased not only because of increases of income, but also because of limited availability of civilian consumption goods—especially durable goods. As a corollary, during the early postwar periods, saving (current saving as well as liquid funds accumulated through wartime saving) was decreased to satisfy deferred demand after government controls were removed.

FIG. 18. NET SAVING OF INDIVIDUALS IN PER CENT OF DISPOSABLE INCOME OF INDIVIDUALS, UNITED STATES

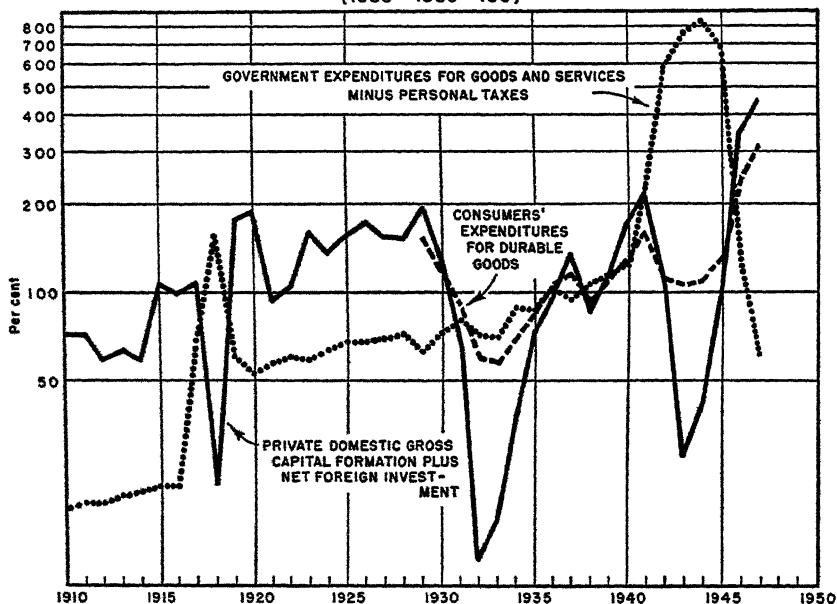


From figure 18 we may conclude that quantitatively significant cyclical changes in the proportion of income saved have been an important stabilizing factor in economic fluctuations. If these changes of saving had not occurred, fluctuations of income and prices would have been more severe. The fact that cyclical changes of saving are not only income-induced, but that the direction of these changes is income stabilizing, makes saving (or consumption) a logically and empirically unsuitable point of departure for an explanation of the cause of income fluctuations. Our attention, therefore, must be focused on changes of investment.

Significance of Investment. Changes of investment are of paramount analytical significance for explaining income changes for three reasons: First, changes of investment may take place spontaneously—that is, they need not be induced by income changes—as are saving and consumption. Second, percentage changes of investment are violent—much more so than percentage changes of consumption. Third, changes of investment are accentuated by sec-

ondary changes of consumption and investment.⁸⁰ Secondary effects upon consumption occur through expenditures of the workers employed in the investment-good industries. Secondary effects upon investment may occur because a large percentage increase in some investment-goods industries requires investment in others. For example, an increase of investment in construction might bring about an increase of investment in industries producing raw materials and machinery used in construction. In contrast, changes of consumption usually do not lead to large secondary effects because, relatively,

FIG. 19. INDICATORS OF INVESTMENT, UNITED STATES
(1935 - 1939 = 100)



even large absolute changes of consumption are small. Such small percentage changes do not require changes of investment in the consumption-goods industries or in the industries producing raw materials for them.

Indicators of Investment. For testing statistically the above reasoning about the processes of income formation in the modern economy, we may differentiate between three indicators of investment (fig. 19): (1) government expenditures for goods and services, minus personal taxes, (2) private domestic gross capital formation, plus net foreign investment, and (3) consumer's expenditures for durable goods. None of these indicators is quite satisfactory in the light of our previous explanation of the meaning of investment. A short discussion of the three series is, therefore, necessary.

Government expenditures for goods and services include items—for example, salaries for the regular civil service—which are not investment. Data

⁸⁰ By some authors these secondary effects of a given investment are called "multiplier effects" as far as consumption is affected and "acceleration effects" as far as investment is affected. The many intricate and controversial aspects of the "multiplier" and the "acceleration" principle need not be discussed here.

are not available to permit separation of such items from investment, such as public construction and armaments. On the other hand, during the two war periods, government expenditures for construction and armaments dominated total investment—that is, public plus private investment. It is not possible, therefore, to disregard government expenditures altogether. The weight of the noninvestment items in our analysis will be kept relatively small for the following reasons: From the standpoint of income formation, that portion of government expenditures for goods and services is of particular interest, that exceeds receipts from personal taxes. We are deducting, therefore, personal taxes from government expenditures for goods and services. The increase of personal taxes reflects fairly well the long-time increase of the noninvestment items in government expenditures. Furthermore, we will be dealing with deviations from trend. The noninvestment items are responsible largely for the upward trend rather than for the fluctuations of government expenditures.

At first glance it appears strange to regard armament expenditures as investment. Suppose, however, we discard the notion that investment must necessarily create productive capital goods—a notion by no means always justified in peaceful lines of investment. At least in the short run, public investment in armaments and private investment in capital goods have the same economic effects. In the long run, as already mentioned, investment in capital goods leads usually (but not necessarily) to increases in productivity. However, under certain conditions, this may be true also for investment in armaments. The quantitative effects upon income formation are generally much greater for changes of investment in armaments than for changes of investment in productive capital goods.

Private domestic gross capital formation plus net foreign investment includes: (1) construction, (2) producer's durable equipment, (3) changes in inventories, (4) net exports of goods and services, and (5) net exports and monetary use of gold and silver. A further breakdown and adjustment of the last two items would be desirable to make the aggregate satisfactory for our analysis. This proved statistically impossible.²⁴ However, this shortcoming is rather insignificant because the relative weight of the last two items in the aggregate is small.

The upward trend in private gross capital formation (including net foreign investment) appears weaker than in government expenditures. To be sure, conclusions with respect to the slope of this trend are somewhat hazardous because statistical data before the 1920's are unsatisfactory, and because, since that time, violent fluctuations dominate the scene. Still, there appears no contradiction for the opinion of those who fear that in secular perspective government expenditures may have to be increasingly relied upon to balance (in the defined sense) the upward trend in the amount of saving indicated above.

Consumer expenditures for durable goods are a less adequate indicator of investment than the production of durable consumer goods. The bias introduced is more one of timing, however, and is not likely to be material. Fluc-

²⁴ As mentioned previously, the old series of national income and product statistics were used before 1929. These series were linked to the new series (available only after 1929) on the basis of their relation from 1929 to 1939.

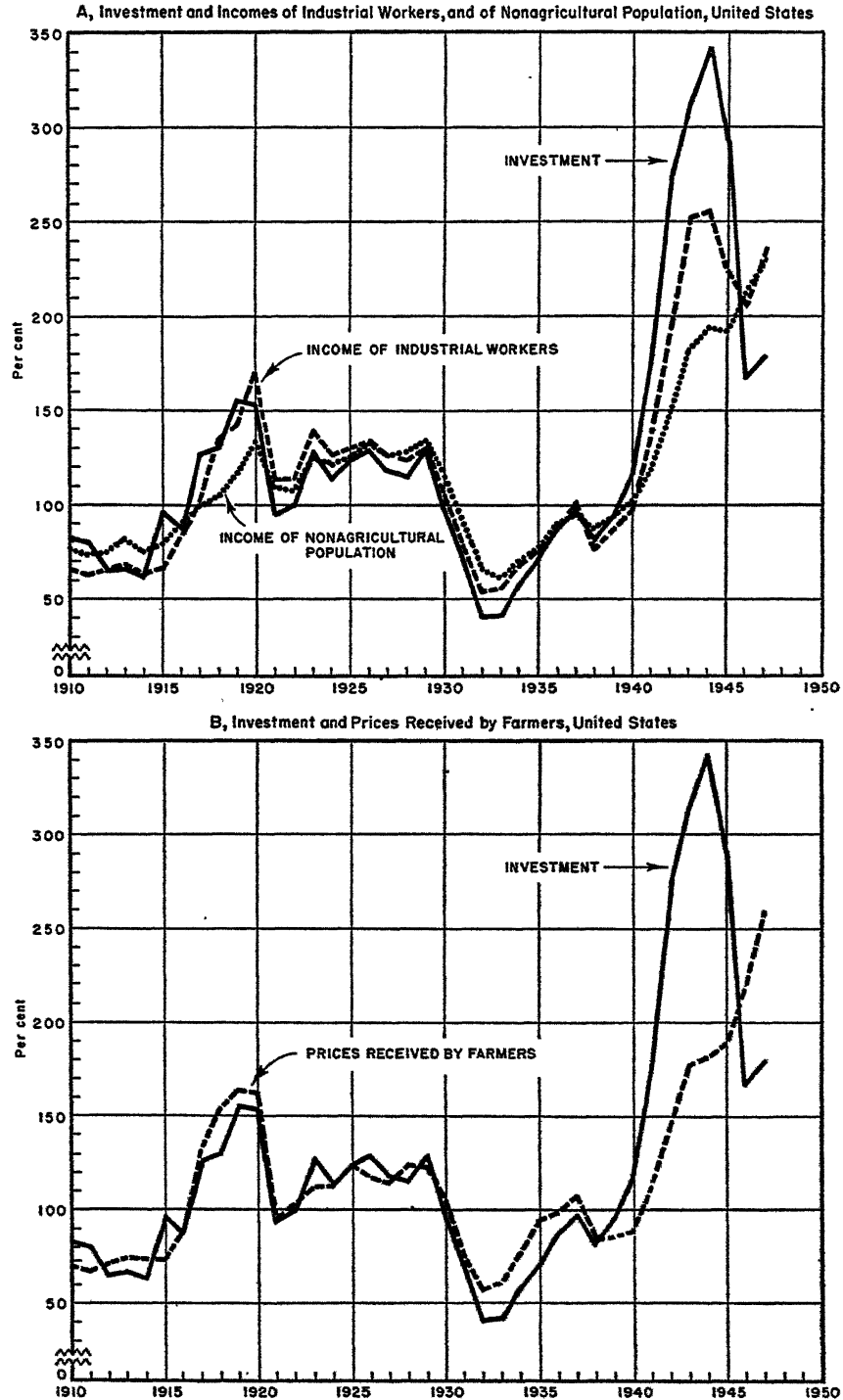
tuations in consumer expenditures for durable goods are less violent than fluctuations in private gross capital formation and sometimes—for example, in 1932 and 1933—lag behind them (fig. 19). Statistically the series is available only since 1929. For this reason and because of the theoretical considerations presented above, it was not included in our composite indicator of investment. A test for the period after 1929 indicated that this exclusion did not materially affect such an indicator.

The question may be raised whether our composite indicator of investment (government expenditures for goods and services, minus personal taxes, plus private domestic gross capital formation, plus net foreign investment) is significant, or whether an indicator of the saving-investment balance would be more appropriate in the light of our previous discussion. The latter indicator can be obtained by deducting personal savings, undistributed corporate profits, and contributions to social security funds from the former indicator. After studying such an indicator of the saving-investment balance in some detail, it was rejected for the following reasons: statistically, the above saving items are not reliable (some of them are computed as residuals) and their definition does not correspond to that of saving in economic theory. More importantly, cyclical variations of personal savings are, as we know, induced by variations of income. Although secular changes of personal savings are, at least in the opinion of many students, independent of income, the evidence on this point is insufficient. Similarly, cyclical variations of corporate saving (that is, undistributed corporate profits) are, partly at least, induced by variations of investment. A theoretical and statistical isolation of these variations from those which are independent of investment proved impossible on the basis of available data. As one would expect, the indicator of the saving-investment balance lagged behind the indicator of investment and showed a considerably smaller amplitude of fluctuations.

Investment and Income. Changes of investment are highly correlated with changes of income (fig. 20, *A*). This correlation is especially high for income of industrial workers. Industrial workers are affected more by changes in the investment-goods industries than the salaried and proprietary groups of the non-agricultural population. For the period 1910 to 1946, the correlation coefficient between fluctuations of investment and those of income of industrial workers was 0.97, and between investment and income of the nonagricultural population, 0.92. The statistical evidence, then, is in fairly good agreement with our thesis that changes of nonagricultural income are primarily caused by changes of investment.

Investment and Farm Prices. In view of these findings, it is not surprising that the correlation between fluctuations of investment and of prices received by farmers is also close (fig. 20, *B*). Before price control, rationing, and subsidies kept agricultural prices under control during and after World War II, this correlation was even closer than the correlation between income and prices (compare fig. 20, *B*, with 17, *B*). As already indicated, price control, rationing, and subsidies obscured and delayed, rather than prevented, the impact of investment and industrial workers' income upon farm prices. The discrepancy between our indicator of investment and farm prices is especially great between 1943 and the first half of 1946. Reasons were given (page 42)

FIG. 20. INVESTMENT, NONAGRICULTURAL INCOME, AND FARM PRICES, UNITED STATES
(TREND = 100)



why, during this period, official price statistics are a poor indicator of the effects of investment fluctuations. If this is taken into account, the statistical evidence is in agreement with the thesis advanced in the preceding section—namely, that many farm products are affected by business activity directly through raw-material purchases and changes of inventories, besides indirectly through nonagricultural income. Investment affects agricultural prices in both ways.

Investment and Foreign Trade. Finally, we may call attention to the relations between fluctuations of investment and fluctuations of foreign trade (section 5). During the interwar period, agricultural exports were closely related to total imports as the main source of foreign purchasing power. Total imports, in turn, are a function of domestic investment, first, directly through raw-material imports by the investment-goods industries, and second, indirectly through income—that is, through imports of raw materials for the consumption-goods industries and through imports of finished luxuries. During the two war periods the close relation between agricultural exports and total imports was broken because special credit arrangements and outright gifts made foreign countries independent of the dollars obtained through United States imports. Still, during these periods the increase of agricultural exports was caused by the same forces—namely, war needs—that caused the increase of domestic investment and income. It is clear now why (as we found in section 5) changes of foreign demand for agricultural products reinforced changes of domestic demand.

In principle, investment fluctuations in foreign countries should also be considered. Practically, for the purpose of this study, this is not essential for three reasons. First, historically speaking, investment fluctuations in industrial countries, which buy the bulk of United States agricultural exports, have been similar to those of the United States:²² changes in internal conditions and external stimuli for investment have generally been international. Second, viewing the future, the weight of the United States economy among the trading nations is so great that international fluctuations of investment are dominated by those in the United States. Third, from the standpoint of agricultural policy in the United States, farm prices and incomes may be shielded relatively cheaply against repercussion of investment fluctuations in foreign countries, because of the much greater relative importance of the domestic market for United States agriculture (section 5); the same reason makes it very costly and politically difficult to shield farm prices and incomes against the effects of investment fluctuations in the United States.



Thus we have seen that unbalance between saving and investment is responsible for changes of nonagricultural income; and that in this unbalance investment changes are the causal factor. Since investment is so important for nonagricultural income, and hence for farm prices, we must ask what factors cause changes of investment.

²² The qualification excludes the U. S. S. R., where investment fluctuations have been of a different nature since World War I. Since 1933, investment fluctuations in Germany have also been different, but the importance of Germany as a market for the agricultural exports of the United States has been relatively small since then.

8. FACTORS AFFECTING CHANGES OF INVESTMENT, AND THE ROLE OF MONEY

Summary. In contrast to agriculture, fluctuations in the investment-goods industries are quantity rather than price phenomena: changes of investment are "real" changes of production and employment. These changes are influenced by two sets of factors which may be called internal conditions and external stimuli, respectively. In principle, both sets of factors are of equal importance for investment fluctuations. Historically speaking, the strength of external stimuli was the essential factor for duration and amplitude of investment fluctuations. Among important external stimuli—for example, technological changes, discovery of new stocks of resources and, especially, war, preparation for war, and postwar reconstruction—monetary factors play a relatively minor role. In investment and price fluctuations, as a whole, money can generally be regarded as a dependent rather than an independent variable.

Quantity and Value of Investment. Investment is defined in terms of value, and the statistics presented in the preceding section dealt with value and not with quantity of investment. It may be well, therefore, to point out that fluctuations of investment are quantity rather than price phenomena (fig. 21).²⁸ Statistics on value of investment, therefore, are also an indication of its quantity. The contrast with the situation in agriculture is striking. As pointed out in section 3, little remains of economic fluctuations in agriculture if the money "veil" is taken off (compare fig. 1 with fig. 6). In investment, on the other hand, economic fluctuations are "real" in the sense that fluctuations of quantity are far more violent than fluctuations of price. As a corollary, cyclical changes of employment occur mainly in the investment-goods industries rather than in agriculture and the consumption-goods industries. We may conclude then that the value of investment is changed because of factors other than the mere change of money funds available for investment. What are such factors?

Factors Affecting Changes of Investment. The rate of investment is determined by two sets of factors. There are, first, certain *internal conditions* in the economic system itself which are favorable or unfavorable to investment—for example, interest rates, wage rates, prices of raw materials, and replacement and maintenance needs for durable goods.

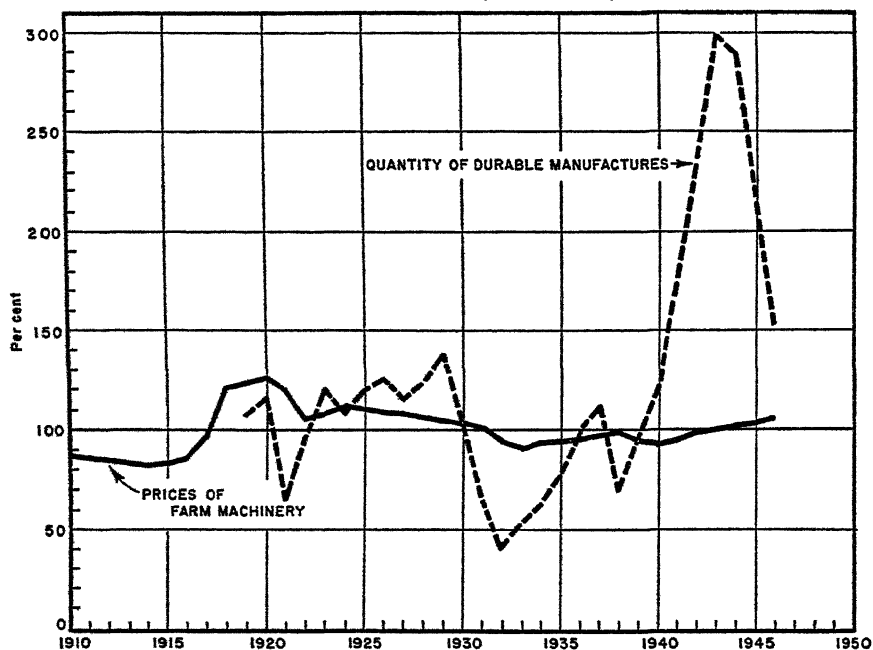
The influence of the rate of interest upon investment is probably greater than the influence upon saving considered above (section 7). However, the influence upon investment is also frequently overestimated. Investors commonly make plans for relatively short periods, let us say for less than ten years. Within such periods, problems of technological obsolescence and other uncertainties are usually more important than interest rates. In the internal

²⁸ A price index of total durable manufactures which could be compared with our quantity index of durable manufactures is not available. We have used, for comparison, prices of that group of durable manufactures in which farmers are especially interested, namely, farm machinery (see also fig. 9, B). Prices of other durable manufactures—as far as they are available—show a similar rigidity.

investment of corporations, interest is imputed but not actually paid out and need not be considered as a factor affecting financial liquidity. Under these conditions, size, prestige, and security of the corporation may become more important considerations for the investment decisions by the management than profitability for stockholders.

The influence of wage rates upon investment is a controversial subject in economic theory. In classical economic theory the responsiveness of investment to variations of wage rates (the elasticity of the demand for labor) plays

FIG. 21. QUANTITY OF DURABLE MANUFACTURES AND PRICES OF FARM MACHINERY, UNITED STATES (TREND=100)



an important role. In recent economic literature the lack of responsiveness is generally stressed. Both points of view may be justified: there are reasons for believing that the responsiveness of investment to variations of wage rates may change (shift) in the course of economic fluctuations. Such a situation makes statistical verification highly difficult. A similar statement holds for the influence of raw-material prices upon investment.

There are, second, *external stimuli* to invest. These may change (independently of changes of internal conditions) the profitability of private investment, or they may consist of circumstances that induce the public to invest irrespective of whether the profitability of private investment has changed.²⁴ Examples of external stimuli are technological changes, discovery of new stocks of resources (among them monetary metals), natural phenomena (harvests, livestock cycles, catastrophies), political changes (affecting eco-

²⁴ This rather general formulation is used in order to avoid such academic terms as "marginal private productivity of entrepreneurial investment" and "marginal social productivity of public investment."

conomic institutions, opening or closing of foreign markets, security of internal and external private investment, and so on) and, finally, stimuli for public investment (peaceful public works, war, preparation for war, and postwar reconstruction).

In principle, internal conditions and external stimuli are of equal importance for investment fluctuations, although in a given historical situation their relative importance may differ greatly. A theoretical explanation of economic fluctuation as a whole based on only one or the other set of factors is not possible. Historically speaking, the essential factor for duration and amplitude of investment fluctuations was the strength of external stimuli. Some external stimuli are always present in a dynamic society. If they are not especially strong, an internal change of sensitivity of the economic system to these stimuli becomes a conditional prerequisite for changes of investment. Changes of internal conditions take place automatically in the course of investment fluctuations. A deficit of investment (in the sense explained in the preceding section), if continued over a sufficient period of time, changes internal conditions so that investment becomes more sensitive to external investment stimuli. A deficit of saving has the opposite effect upon internal conditions. On the other hand, if external stimuli are very strong, investment activity may continue at a high level even though internal conditions have become increasingly unfavorable. In such a situation, contraction of investment activity is usually very severe after external stimuli have weakened.

A detailed analytical and historical discussion of internal conditions and external stimuli is beyond the scope of the present study.⁵⁵ Among internal conditions, changes of price relations probably rank first. The most important external stimuli are technological changes, discovery of new stocks of resources and, especially, war, preparation for war, and postwar reconstruction.

Role of Money. The role of another factor may be explored here somewhat further, because throughout the history of the United States it has attracted the interest of farmers as an explanation and as a remedy for fluctuations of agricultural prices. This factor is the monetary system and, especially, the quantity of money. Money may have effects upon prices. These effects are taken into account under internal conditions and external stimuli for investment.

Among internal conditions of investment, the quantity of money is most directly related to credit conditions—that is, to the interest rate and credit rationing. Such effects of changes in the quantity of money upon investment should not be overestimated for two reasons: First, factors other than changes of interest rates are usually more powerful in altering the profitability of private investment.⁵⁶ Second, at low interest rates, as at present, an increase (but not necessarily a decrease) in the quantity of money has little effect upon interest rates. At low interest other internal conditions for investment—for example, wage rates and raw material prices—become relatively more important for investment than interest rates.

The negative functional relation between quantity of money and interest rates is a curve that flattens out at low interest rates. The reason is as follows: Interest rates are gross rates which include allowances for risk, uncertainty,

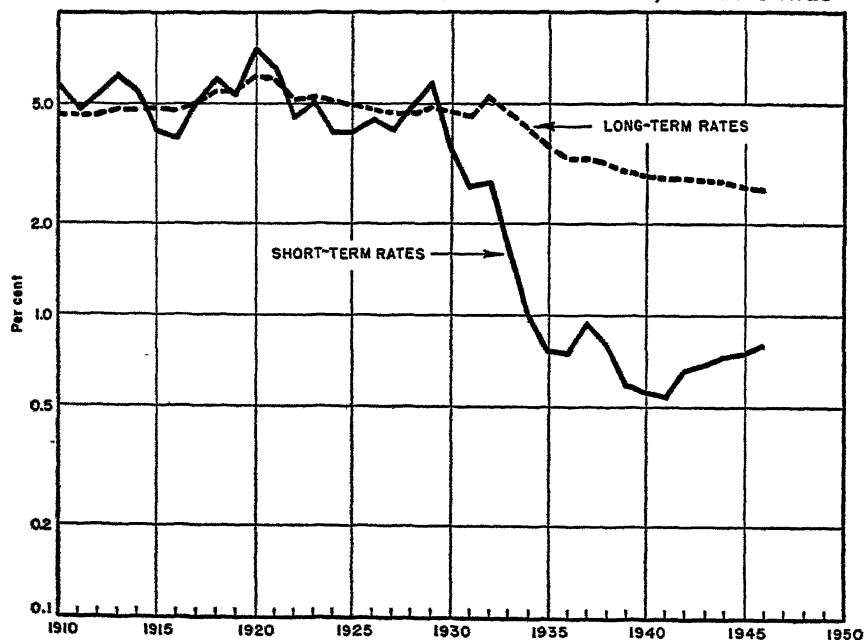
⁵⁵ For a detailed analytical and historical discussion of the interrelation between the two sets of factors, see Ciriacy-Wantrup (1936, 1938a, 1938b).

⁵⁶ For a historical analysis of this point, see the literature cited in the preceding footnote.

costs of lending, and income taxes. If the minimum gross rate represented by these items is approached, an increase of loanable funds through credit creation does not decrease the rate further. The minimum gross rate differs for long-term and short-term loans, as illustrated by figure 22.

Among external stimuli of investment, the purchasing power of monetary-metal producers is, at present at least, small in relation to others—namely, the pent-up domestic demand for consumer and producer durable goods, foreign needs for relief and rehabilitation, and continuing requirements of the military establishment.

FIG. 22. SHORT-TERM AND LONG-TERM INTEREST RATES, UNITED STATES



More important than the cause-effect relations just discussed is the opposite one: in a correlation with investment and prices, the quantity of money must generally be regarded as a dependent rather than an independent variable. This is quite obvious for bank money in a monetary system like that of the United States. Within the wide and flexible limits of reserve requirements,²⁷ money can be readily created and canceled in response to fluctuations in demand for money to finance investment. This is especially true when the demand for money originates from the investment needs of the government. In this case, reserve requirements become largely nominal. In the field of public policies, effective action with respect to economic fluctuations requires a combination of monetary and fiscal measures, especially the latter (section 9).

Dependence of the quantity of money on investment and prices is not confined to bank money, but occurs also in the production of monetary metals.

²⁷ These requirements are flexible because of actual changes in the requirements and because of open-market operations of the Federal Reserve System.

First, given certain technical possibilities of production and prospecting, the actual rate of gold and silver production is influenced strongly by the prices of productive services used. Second, money metals are to some extent joint products with other metals—for example, copper—which are important raw materials for the durable-goods industries; fluctuations in the production of durable goods, therefore, also lead to fluctuations in the production of money metals. Third, external stimuli to invest—for example, technological advances in metallurgy, opening and closing of foreign markets, wars—may also have important effects upon the production and distribution of monetary metals.

The foregoing hypothesis about the cause and effect relations between investment and prices, on one side, and the quantity of money, on the other, may now be compared with available data. Fluctuations of the quantity of money may be correlated with fluctuations of investment (fig. 23, *A*) and of prices received by farmers (fig. 23, *B*).³⁸ Fluctuations of the quantity of money were less pronounced than fluctuations of investment and farm prices, and at some important turning points lagged behind them. Annual data are inadequate for an analysis of turning points. This problem, therefore, was investigated on the basis of monthly and quarterly data as far as available. In 1915, 1919, 1921, 1929, and 1933 the movements of investment and of seasonally adjusted prices received by farmers (and of wholesale prices) preceded movements in the quantity of money. The same holds true for production and employment, although data in these fields are inadequate. During the less important turning points in 1937 and 1938, prices lagged. In these years, however, harvest fluctuations and the foreign political situation created rather erratic movements. Thus, statistical data do not point to fluctuations in the quantity of money as playing a primary initiating role in fluctuations of investment and farm prices.

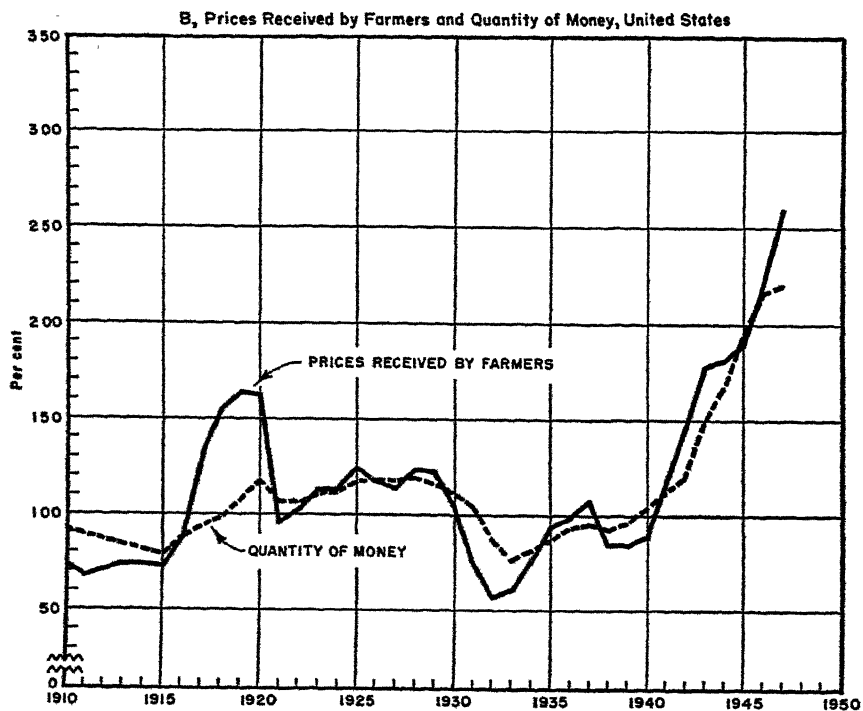
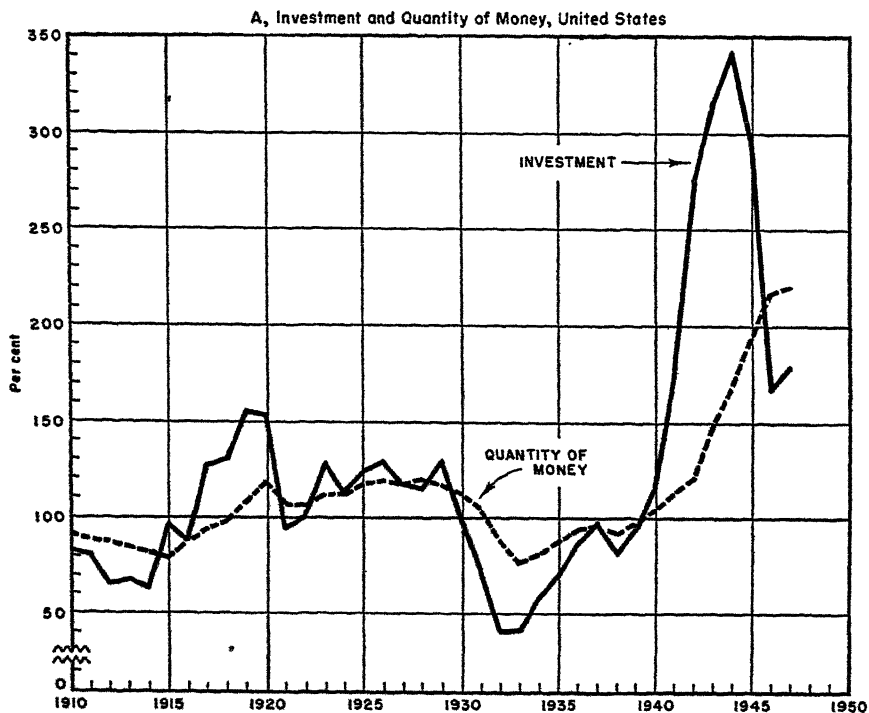
It may be objected that, before drawing this conclusion, we should consider not only the quantity of money, but also the velocity of its circulation. Small analytical significance attaches to any explanation of price fluctuations through velocity of money. Velocity merely becomes a catch-all for internal conditions and external stimuli of investment and for other factors which themselves remain in the dark. Aside from this objection, recent statistical studies (Warburton, 1945; Fisher, 1945) seem to indicate that income velocity of circulation (after correction for its downward trend, after statistical adjustments, and after excluding "speculative" transactions and the last extreme stages of inflation as in central Europe after World War I) is rather stable instead of very unstable, as commonly imagined.



What significance do these findings have for public policy and private action? At what stage or stages in this chain of relations between internal conditions and external stimuli of investment, on one side, and farm prices

³⁸ Total deposits (that is, time deposits plus demand deposits adjusted for interbank and United States government deposits less cash items in the process of collection) plus currency is a more significant indicator of the quantity of money than demand deposits plus currency, because time and demand deposits are substitutes for many important purposes; furthermore, statistical differentiation between demand and time deposits has not always been uniform.

FIG. 23. INVESTMENT, QUANTITY OF MONEY, AND PRICES RECEIVED BY FARMERS, UNITED STATES (TREND = 100)



and incomes, on the other, will public measures be most effective in reducing the extremes of economic fluctuations? And how can an understanding of these relations help a farmer adjust his program to cushion the effects of economic fluctuations on his business? An attempt will be made to answer these questions in the following section.

9. CONCLUSION: SOME IMPLICATIONS FOR PUBLIC ECONOMIC POLICIES AND FOR INDIVIDUAL ACTION

Agricultural Programs and Anticyclical Policies. Traditionally, the concern of farmers is more with agricultural programs than with policies directed toward stability of investment and nonagricultural income. The preceding sections tried to show that farmers, in California as elsewhere, have a real stake in the latter problem. The economic position of farmers, as a whole, is influenced more by fluctuations of investment and nonagricultural income than by parity-price legislation, agricultural production controls, agricultural tariffs, an ever-normal granary, or by other more narrowly agricultural policies to which farmers and their political and academic representatives are giving much attention.

Concern for their own economic position is not the only reason why farmers have a stake in economic stability. Farmers want to play their part as good citizens in maintaining social institutions that guarantee a maximum of individual freedom and opportunity for all. Economic instability is an important threat which endangers such institutions. The progress in organization and political influence which farmers have made during the last decade imposes upon them the responsibility to look beyond their line fences toward the welfare of the commonwealth.

Public versus Private Investment Decisions. It was shown that an increasing rate of investment, to balance (in the sense explained in section 7) an increasing rate of saving,²⁰ is the crucial problem of economic stability in a modern dynamic society. We also observed that, in the past, investment was partly private and partly public, with the latter share increasing. This pattern will be assumed here to continue also in the future.

Division of the power over investment decisions between private enterprise and the public is not the only possible assumption. Solutions of problems created by unbalance of saving and investment would be facilitated if all investment decisions were made by the public. However, the problems of long-run efficiency of an economic system are different from those of its cyclical stability. From the strictly economic standpoint, a socialized economy is probably superior only in solving the latter problems. Moreover, from the standpoint of maintaining free social institutions (in the above sense), division of power over investment decisions between private and various (federal, state, local) public interests appears as an essential safeguard, against both private monopolies and a totalitarian state.

Under the assumption of such a "mixed" economy, then, we may consider internal conditions and external stimuli of private investment and some public economic policies relating to them. Let us start with internal conditions.

²⁰ In the sense of annual amount of income saved, not annual proportion of income saved.

Price Structure. At present (spring, 1947), a considerable distortion exists in the price structure. Agricultural prices, the flexible portion of raw-materials prices in general (including lumber, paint, and other materials used in construction), and industrial wage rates are considerably higher than their long-time trend relation to other prices, especially those of durable goods, and of raw materials characterized by rigid prices (for example, steel, fertilizer). Some distortion of this kind is quite normal at the height of an upswing (section 4). However, this distortion is greater now than in 1920 and 1929. At the 1920 turning point of investment fluctuations, the worsening of internal conditions for investment was partly due to the rise of interest rates. At present, interest rates are tightening but are still low.⁴⁰ However, the existing distortions just referred to, the satisfaction of deferred demand for nondurable consumer goods, and the completion of the process of filling up inventories, will eventually bring about a decrease of investment, income, and prices similar to that in 1920-1921.

Inflationary Tendencies. Until this occurs, dangers threaten from the opposite direction—that is, from an excess of investment over its equilibrium rate and from resulting inflationary tendencies (section 7). These dangers are not discussed here in detail because they may have passed when these lines reach the reader. The reasons for this hope are: (1) A balanced federal budget or even a small budget surplus has removed the most important factor responsible for inflationary potentialities. (2) The recent survey of liquid assets, previously mentioned, has shown that a too abrupt liquidation need not be feared. (3) Production is at last getting into full swing in spite of social friction and technical bottlenecks. (4) Inventories are being filled. (5) The increase of prices after removal of O.P.A. already reflects a part of the postwar decrease in current and accumulated saving. However, political developments requiring increased public investment for foreign aid and for the military establishment may offset these favorable factors.

Even if a temporary decrease of private investment occurs, a quick and decisive revival and a check of price decreases—similar to that between 1921 and 1929—appears possible. The main reason for such a possibility is the existence of external stimuli for private investment which, as explained below, will probably remain rather strong during the first post-World War II decade even after the deferred demand for nondurable consumer goods and inventory repletion has been met.

Two Questionable Public Policies. Two public policies may impede the correction of the existing distortion of price relations during a temporary recession of private investment. For the time being, the result may not seriously interfere with a revival but may be of importance for a severe depression of private investment later on.

First, many raw materials are of agricultural origin. Through parity legislation the prices of agricultural products are tied to the more rigid (see section 4, especially figures 14 to 19) prices of commodities farmers buy. Technically and politically, parity prices (or income) for agriculture have

⁴⁰ Public policies are in favor of "cheap money" in order to facilitate public borrowing, and to ease the public interest burden. Official support of the market for federal bonds makes the latter virtual substitutes for money instruments. Quantitatively, private holdings of federal securities loom large in relation to money in the narrower sense.

become generally accepted. Continuation of these policies in some form may be expected after present government commitments have expired on December 31, 1948.

Second, leaders of labor unions—whose decisions have become one of the most important factors in the economic life of the nation—some government officials, and some academic economists are apparently in favor of a policy of maintaining and of possibly increasing wages during depressions “to maintain purchasing power”—besides pushing wage increases during prosperity under the “ability-to-pay” and “cost-of-living” principles.

There are several economically unsound aspects of the parity-price concept with which we are not concerned here—for example, the historical base, the disregard for secular changes in technology and consumption patterns, and, accordingly, the undesirable effects upon resource use and income distribution. We can only consider those aspects of parity which have to do with economic stability.

If parity prices are obtained through production controls to maintain agricultural prices (not through payments by the federal treasury), such controls interfere with what we called the automatic changes of internal conditions in the course of investment fluctuations (section 8). During the upswing, farmers reap the advantages of flexible prices (section 4). It is human nature that these advantages are quickly forgotten during the downswing. Farmers want to obtain through the parity device the advantages of rigid as well as flexible prices. This attempt has the effect of preventing the improvement of internal conditions for investment, which otherwise would take place during the downswing through decrease of raw-material prices, food costs, and wage demands.

Wage rates have maintained for a long time a strong upward trend. The sound economic basis for this trend is the increase in labor productivity. Historically, demand for labor during prosperity has forced expression of increases in labor productivity through wage increases rather than through price decreases. In recent decades, union policy and monopolistic elements in industry have accentuated this historical tendency. During depressions, average labor productivity decreases because of decreases in the volume of business. At such times, wage increases discourage employment in the investment-goods industries further. The effect on income and prices may be greater than that of wage increases on the purchasing power for consumption goods.

The question may be raised how serious the worsening of internal conditions through the two policies are at the present time. Although the elasticity of demand for raw materials and labor for investment is generally not great, it probably changes in the course of economic fluctuations (section 8). This elasticity is probably not negligible when external stimuli for investment are weak. As long as external stimuli remain strong, as can be expected during the next decade, private investment may revive even if a temporary recession does not fully correct the existing distortion in price relations. However, under these conditions, the eventual decrease in investment and prices will be severe when external stimuli weaken.

Subsidies to Consumption or to Investment? Another relation between the two policies and economic stability is of greater theoretical and practical significance.

If parity prices (or income) are obtained through payments by the federal treasury (not through programs to maintain agricultural prices), the effects just discussed need not occur. Nevertheless, the relation between parity payments and economic stability remains important. At first sight a subsidy to agricultural income during a depression may appear desirable to stabilize or to increase the total income stream of the economy. What is the validity of such an argument?

Agricultural production, as we know (section 3), is rather stable. A subsidy to agricultural income therefore, is largely a subsidy to consumption, saving, and land values rather than to investment. After a prolonged depression, deferred replacement and maintenance needs accumulate in agriculture, as in other industries. Under these conditions, a subsidy to agriculture may become a subsidy to investment. This is not true in the beginning of a depression—the time when public anticyclical policies should be applied. Furthermore, such policies cannot be confined to assistance in filling replacement and maintenance needs. Usually, new investment outlets must be created.

To subsidize saving is, on the basis of our previous discussion, abortive from the standpoint of stabilizing a decreasing income stream. To subsidize consumption is a relatively (compared with a subsidy to investment) ineffective way to increase the income stream.⁴ This is as true for parity payments or other subsidies in agriculture as for wage increases (greater than warranted by increased labor productivity) in industry. The reason was already mentioned (section 7): because of the large total value of consumption (relative to investment), a practically conceivable subsidy, let us say of four billion dollars per year, would be a rather modest percentage increase of consumption, and consequently would lead to small, if any, secondary effects (section 7) upon employment, investment, and consumption.

A four-billion-dollar addition to consumption would amount to less than 10 per cent of consumers' expenditures for goods and services during the depth of the "great" depression 1932–33, and less than 5 per cent during recent years. Year-to-year and even seasonal fluctuations of consumer expenditures of these magnitudes are common. An addition of this magnitude would require little additional employment and investment in the consumption-goods industries and certainly none in the investment-goods industries.

In the short run, furthermore, the "leakage" into saving from income subsidies must be considered; as we know, the proportion of income saved tends to increase with increasing incomes, especially in the beginning of income increases (section 7).

On the other hand, if the same amount of subsidies were used to increase investment, the stimulus would be very great, and would lead to large secondary effects upon employment, investment, and consumption. Such subsidies could be made either directly through a public-works program or indirectly through public assistance to private construction in the fashion of the F.H.A.

A four-billion-dollar addition to investment would amount to approximately twice the total private construction expenditure (residential and industrial) in the depression years from 1932 to 1935; it would amount to

⁴ We are considering here consumption subsidies to increase the total income stream. Consumption subsidies to raise nutritional standards—for example, through a food stamp plan, through school lunches, soup kitchens—are economically and socially desirable.

approximately half of private construction during the prosperity years from 1927 to 1929 (during recent years private construction was at depression levels because of wartime restrictions). An addition of this magnitude would not only be a great stimulus to employment and investment in construction but also would require additional employment and investment in industries producing machinery, tools, and raw materials for construction.

As was already pointed out (section 7), investment has a double aspect: it increases the income stream, and usually (but not necessarily) it increases productivity. The latter aspect, which is frequently forgotten when income-creating policies are discussed, is another reason why subsidies to investment are generally more desirable than subsidies to consumption.

The Future of Investment. The problem just considered, of whether public economic policies can influence the income stream most effectively through aiding consumption or investment, leads to the question whether there will be any need for public aid to investment, and, if so, what form it should take.

Whether in secular perspective, private investment will or will not keep up a balance with saving is partly a matter of the general institutional and political climate under which private enterprise has to operate. This climate now is not as favorable as in the beginning of the 1920's, and the future is somewhat cloudy.⁴² If private initiative is curtailed for political and institutional reasons, the government must take over a larger share of investment decisions. Regardless of whether one wishes or fears such a development, one cannot dispose of it simply by reference to the "refusal" of private enterprise to take risks, or to the "insufficiency" of investment opportunities.

In Secular Perspective. A secular forecast of external stimuli for investment contains no small speculative element. Although private gross capital formation did not exhibit a strong upward trend during the last two decades (fig. 19), it has not been convincingly demonstrated that the pace of technological change, of the discovery of new resources, and of the opening of new markets, has slackened. To be sure, in some parts of the world, particularly western Europe, the United States, and possibly Japan, the rate of population growth has decreased (even here the evidence is not easily interpreted). However, other areas, notably eastern Europe, Russia, and parts of South America, are still in the midst of the great increase in the rate of population growth which takes place in the earlier stages of industrialization. Other parts of the world, especially China, India, Indonesia, and parts of South America, have not yet experienced the full impact of industrialization.

The relation between population growth and investment is by no means simple. After a certain population density is reached in a given country, the growth of real income per person is more important for stimulating and absorbing investment than population growth. As a corollary, it is possible to have, temporarily at least, a rapid growth of population without rapid growth of investment because savings are hoarded and because industrial and managerial skills are scarce. The result is a stationary (if agricultural production can be expanded) or decreasing real income per person and ultimately a reassertion of the Malthusian law. Examples are found in colonial and semi-

⁴² This broad but important factor cannot be treated in detail within the space allotted to this study. The reader is referred to Schumpeter (1942). For proper balance, the excellent review article by Hardy (1945) should be read in conjunction with this book.

colonial areas where mortality in early childhood has been reduced through the influence of western science. In general, changes of population growth must be regarded as a dependent rather than an independent variable in changes of technology and investment. This is true not only for secular changes in population growth just considered, but also for cyclical changes of population growth—which are of rather small amplitude (Ciriacy-Wantrup, 1936, 1938*a*, 1938*b*).

In the Intermediate Future. Whatever one's opinion may be with respect to the secular prospect for external investment stimuli, for the intermediate future—let us say the next decade—one does not need to be pessimistic. Let us assume for our appraisal that it will be possible to maintain peace for the next decade—at least a peace of that precarious nature that prevailed between 1933 and 1939.

At home, war-deferred needs of consumer durable goods, especially houses, can scarcely be satisfied in less than a decade. Estimates of the country's housing need range between eleven and sixteen million units. New home construction is at present at an annual rate of about one million units. Deferred maintenance of industrial plants and equipment will likewise require several years. In addition, the war has greatly accelerated technological obsolescence in existing industries, has given great impetus to new industries (in the fields of aviation, light metals, synthetic rubber, synthetic fibers, plastics, wood products, radar, and radio) and has laid the foundation for other industries still in the initial stages of development but with the promise of great future importance (in the fields of jet propulsion, rockets, synthetic fuels, and atomic energy). Furthermore, it should not be forgotten that the relatively small volume of private gross capital formation during the pre-World War II decade will have some importance for future investment needs. This situation is quite different from that which prevailed after World War I. Already, capacity in some basic investment-goods industries—for example, steel—is regarded by some as insufficient.

Abroad, the war has not only created a backlog of demand for durable goods and accelerated the pace of technological change but, in contrast to the United States, has destroyed durable goods on a large scale. More importantly, and this is quite different from World War I, important durable-goods industries themselves have been destroyed during the war, or their productivity is being paralyzed through "peace" policies, especially in Germany and Japan. Political changes and the experience that only industrial countries can hope to fight a modern war successfully, and to raise standards of living appreciably in peace, have increased the demand for industrialization all over the world.

Some of these investment stimuli—for example, those connected with capital exports and atomic energy—will require public participation. There are others which primarily stimulate public investment, but which have considerable secondary effects upon private investment. Among them, one may mention slum clearance, superhighways, resource conservation and development, greater social services in the fields of health, nutrition, recreation, education, old-age security, and, most importantly, a much larger peacetime military establishment. With respect to the latter, as has already been indi-

cated, the future will probably have greater similarity with the situation prevailing between 1933 and 1939 than with the 1920's.

The Need for Public Policy. Existence of these and other investment stimuli does not assure that investment will increase at a stable rate. On the contrary, it can be expected that private investment in the future will be no less erratic than in the past (fig. 19, p. 48). There are two reasons for such an expectation: The first reason is the present great boom in private investment. The abnormal concentration of private demand for durable goods during the post-war period will in itself generate fluctuations of this demand in the future. Second, because of the political implications of some important external stimuli and because of changes in the institutional climate already emphasized, private investment will probably be rather sensitive (if not "nervous") with respect to political changes at home and abroad. Under these conditions public economic policies to stabilize the flow of investment appear necessary.

The Goal of Public Policies. The goal of public policies can scarcely be avoidance of investment fluctuations altogether. Such a goal would not only be overambitious, but dangerous. It would require positive anticipating actions, which, in view of the great uncertainties surrounding any forecast of private investment, would almost certainly lead to serious mistakes. An illustration of such mistakes is found in the experience with public policies put into effect to avoid the depression which many economists and government officials expected right after the end of World War II. The political implications of anti-cyclical economic policies are great. There is a temptation to make forecasts fit (consciously or unconsciously) into a political pattern that appeals to the forecaster. This danger becomes the greater the more ambitious the goal set for public policies.

The danger just indicated is not reduced through the use of "economic models" for forecasting, a use which has become rather popular since World War II. Such models mean merely that a hypothesis is spelled out in quantitative terms. Many users of these models forget that the essential problems are soundness and verification rather than the mere quantitative restatement of the hypothesis. Such quantitative restatement may lead to dangerous simplifications, omissions, and distortions by those who are mainly interested in the deceiving appearance of security that figures give, rather than in the soundness of the underlying hypothesis. Unfortunately, a shining quantitative model is better suited to sway public opinion or to cover up nonscientific motives than a clear and detailed statement of the hypothesis.

Fiscal and Monetary Policies. During the boom of private investment, the following modest and negative but practical and safe goals of public policy may be set:

1. Deferment of the greatest possible amount of public capital expenditures. However, not all public capital expenditures are deferable. National security and public health and safety would seem more important than economic stability. Other capital expenditures—for example, dam construction—cannot be discontinued for technical reasons after having been started.

2. Increase of public revenues through keeping tax rates as high as feasible without decreasing incentives to individual effort. This is especially true for pay-as-you-go taxes (personal income, payroll, sales, and turnover taxes). In

the interest of ease and speed of rate adjustment, the problem of progressiveness of taxes should not be tied up with that of anticyclical effects of taxes.

3. Cessation of public borrowing, increase of liquid reserves, and, under certain conditions, increased amortization of the public debt. The advisability of increased debt amortization depends on the type of debt (short-term, long-term) and of the holder (banks, institutions other than banks, corporations, and individuals).

4. Prohibition or restriction of a large flow of credit into the purely speculative markets (stock and commodity markets, real estate). Such a flow was partly responsible for the excesses and the sharp reaction in 1929. Generally, however, not too much should be expected from this measure in dampening a boom.

All these measures may be characterized as economic preparedness for a depression. Besides, they help to avoid the excesses of a boom. They do not commit the government to take positive, anticipating action to prevent a depression.

If the fiscal measures suggested under the first three points are employed sufficiently early and resolutely, it may not be necessary to tighten credit conditions through more strictly monetary measures (changing reserve requirements and discount rates, open market operations, and so on). Frequently, however, fiscal action must be supported by monetary action when "full" employment is approached. As unemployment approaches a low level, greater and greater increases of income and prices are necessary to absorb an additional amount of unemployment. This situation requires close watching by fiscal and monetary authorities. It is an oversimplification if "full" employment is stated in one numerical term. The problem is essentially one as to how much increase in prices should be tolerated to decrease unemployment.

In the beginning of a decline of private investment, some general relief in tax rates may be given. Main reliance, however, would probably have to be placed on public capital expenditures and, at least after internal conditions for investment have become more favorable, on public assistance to private investment through loan guarantees, specific tax concessions, and direct subsidies. These activities make budget deficits and, if reserve funds are exhausted, an increase of the public debt unavoidable.

Public Debt. How far an increase of the public debt is not only of cyclical but also of secular nature—that is, not offset by debt reduction during prosperity—depends on the measures of economic preparedness suggested for the period of prosperity and on the problems of secular growth of private investment already referred to. An anticyclical economic policy properly designed with respect to timing and volume of taxation and borrowing does not necessarily involve a rapid secular growth of the public debt. On the other hand, before becoming alarmed about a secular increase of the public debt and its results upon the income distribution, one must compare the increase in the interest burden with the increase of the national income (including collective items such as free education, medical care, recreation, and transportation) and changes of indebtedness in the public sector of the economy with those in the private sector. As long as the public debt is an internal debt—not owed to foreign countries—there is little reason for alarm as long as the interest

burden does not increase faster than the national income and a "rentier class" does not become too large, too fixed, and politically too powerful. The last danger appears rather small in a democracy, provided proper attention to this problem is given in the methods of taxation and borrowing.

Type of Public-Investment Outlets. There are two aspects of properly designed and executed fiscal policies which are of special interest to agriculture—aside from the farmer's interest in greater stability of the whole economy. These aspects are connected with the type of public-investment outlets and the type of taxes which fit best into such policies.

Conservation Policy. It is the aggregate of public and private investment that counts. Hence, public investment should choose outlets that will make it complementary to and not competitive with private investment. Investment outlets which satisfy this condition are largely in fields where returns are collective or are extra-market goods and services. One such field of special interest to farmers is the conservation of natural resources, especially of soil and water. Public expenditures for conservation can well be integrated into an anticyclical fiscal policy; furthermore, the economic possibilities of private expenditures for conservation are increased by greater economic stability (Ciriacy-Wantrup, 1946).

Taxation Policy. It was shown in section 4 that the rigidity of real estate taxes is an important cause for the worsened economic position of farmers during depressions. For the American farmer, real estate taxes in the form of the general property tax are more significant than any other type of taxes. In the United States, for instance, it is estimated (1927) that 83.8 per cent of all federal, state, and local taxes paid by agriculture were general property taxes (Coombs, 1930). During the depression years in the thirties this percentage was probably even higher. During the recent war years it was lower.

On the other hand, it was just pointed out that an anticyclical fiscal policy requires flexibility of taxes and main reliance on pay-as-you-go taxes. A shift in emphasis from the property tax to income and sales taxes with rate adjustment during prosperity and depression would generally be to the advantage of farmers (Ciriacy-Wantrup, 1944).

There are some institutional obstacles to overcome in making the proposed shift in taxation. Support of local governments (counties, cities, special districts, in some cases, states) depends upon the general property tax. Administratively it would be a great economy to levy local taxes as a percentage of federal income taxes; from the standpoint of over-all tax economy it would thus become worth while to refine administration of the latter to a very high degree. Such a tax reform—which, of course, could be brought about only gradually—would result in greater fluctuations of local tax receipts. As we know, it would be highly undesirable to balance the decrease of the tax base during economic depressions through increases in tax rates. One remedy would be to use reserve funds already mentioned to stabilize revenues available to local governments for expenditure. Another would be to facilitate borrowing and debt retirement by local governments during economic fluctuations. In this way local governments could effectively supplement federal anticyclical fiscal policies. Both remedies require new social institutions which can scarcely come into existence and effective operation without federal assistance.

Farmers and their political representatives can help a great deal to bring such reforms closer to realization.

What the Farmer Can Do Himself. Finally, we may consider briefly some measures which the individual farmer himself may undertake, to guard against the effects of economic fluctuations. The goal for such private measures is not unlike the goal set above for public policies: the best defense against the effects of a depression is economic preparedness during prosperity. What does such economic preparedness mean for the individual farmer?

1. He should keep himself well informed about the national outlook for non-agricultural business activity. Such information should receive prominent attention in the reports of public and semipublic agencies which supply outlook material.

2. During prosperity the farmer should attempt to decrease fixed charges (section 4). The problem of taxes has already been considered. There remains the problem of the interest burden. The increase of net income during the upswing should be used first for decreasing long-term debts. No new long-term debts should be contracted.

3. Income disposable after elimination of long-term debts may at least in part be accumulated as liquid reserves in United States saving bonds or similarly safe and stable securities. What percentage of disposable income is saved in this form is determined by the advisability of investment (see the next three points) and by the income tax. It is well known that, for purposes of the income tax, farmers have some legitimate opportunities to charge investment to current expenses. Frequently this may be more advisable than saving because of the resulting lowering of income taxes.

4. The timing of saving and investment deserves consideration. Land should be purchased in the beginning of the upswing before land values have materially increased. After land values have increased, disposable income is better accumulated in liquid reserves. During the depression such reserves may be profitably used for buying land at lower prices. The same timing is not advisable for the purchase of equipment. Prices of farm machinery are rather rigid during economic fluctuations (section 4). It is usually profitable to invest in farm machinery during prosperity in order to decrease hired labor costs. Farm wage rates are rather flexible during economic fluctuations (p. 25).

5. The investments that should receive priority during prosperity are those that decrease recurrent cash expenditures. Such a decrease helps greatly in weathering the depression. For example, a farmer who rents some of his acreage may well purchase land (but see the preceding paragraph) provided he has cash available, and does not merely exchange the obligation to pay rent against the usually more rigid obligation to pay interest. The same is true for improvements in buildings and equipment to reduce costs of hired labor and of upkeep.

6. During prosperity, particular attention should be given to the problem of soil depletion. The fertilizer budget should be expanded. As we know (section 4), fertilizer prices belong to the rigid portion of the price structure. The farmer should take advantage of this during the upswing. Other soil-conservation measures requiring cash expenditures should likewise be under-

taken during prosperity. In principle, this point aims at the same end as the preceding one. But the importance of maintaining and, if possible, increasing soil productivity during prosperity is so great that special emphasis appears justified.

Without fixed-interest charges, with liquid reserves, with efficient equipment, and with well-maintained soil productivity, the farmer's economic position in entering a depressing is much stronger than that of other classes of the population—for example, industrial workers, most business men, and dividend recipients. The farmer need not fear unemployment (section 3). His real income from home-produced food and shelter remains unimpaired. To be sure, prices received will decline relative to prices paid (section 4). But if the suggested measures of economic preparedness were taken during prosperity, the farmer can reduce cash expenditures for a considerable period of time without too great a decrease of productivity. This is particularly true for the family farmer.



We may conclude, therefore, that under proper anticyclical public policies and proper private measures of preparedness, economic fluctuations are no valid reason why farmers should become special wards of the government. Farm-relief measures in existence or proposed by agricultural economists, such as parity legislation and various forms of general subsidies—that is, subsidies not aimed at specific social objectives such as resource conservation, better nutrition, and specific adjustments of production—are a superficial, and in the long run, ineffective attack upon some symptoms of economic fluctuations. Such policies deflect the attention of farmers and of the public from those general anticyclical public policies and those private measures of economic preparedness which are able to take most of the danger—to individual farmers and to social institutions—out of economic fluctuations.

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Fig. 1.: United States cash farm income:

1910-1923: U. S. Department of Agriculture. *Agricultural Statistics, 1942*, p. 660.

1924-1942: U. S. Bureau of Agricultural Economics. *Cash Receipts from Farming by States and Commodities, Calendar Years, 1924-44*, p. 158-60. Washington, D.C., 1946. Processed.

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California cash farm income: Same as above except:

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Fig. 2.: United States farm population:

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Fig. 3.:

A, Livestock and livestock products: Same as figure 1.

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D, Vegetables, United States cash farm income:

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Fig. 4.: Same as figure 3.

Fig. 5.: Same as figure 1 and the following:

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Fig. 6.: Same as figures 1 and 5.

Fig. 7.:

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Fig. 8.: Same as figure 7 and the following:

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Fig. 9.: Same as figure 8.

Fig. 10.:

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Fig. 11.:

Value:

- 1895-1899: U. S. Department of Agriculture. *Yearbook, 1910*, p. 674.
- 1900-1909: U. S. Department of Agriculture. *Agricultural Statistics, 1941*, p. 481.
- 1910-1945: U. S. Department of Agriculture. *Agricultural Statistics, 1942*, p. 537. And U. S. Office of Foreign Agricultural Relations. *Foreign Agricultural Trade, United States Foreign Trade in Agricultural Products*, Apr., 1946, p. 8, and Dec., 1946, p. 8. Processed.

Quantity:

- 1895-1914: U. S. Foreign Agricultural Service. *Foreign Crops and Markets*, vol. 31, no. 9, p. 263-64. Aug. 26, 1935. Processed.
- 1915-1939: U. S. Department of Agriculture. *Agricultural Statistics, 1942*, p. 540.
- 1940: U. S. Bureau of the Census. *Statistical Abstract of the United States, 1944-45*, p. 655.
- 1941-1944: U. S. Office of Foreign Agricultural Relations. *Foreign Crops and Markets*, Annual supplement, Aug. 31, 1945, p. 5. Processed.
- 1945: U. S. Office of Foreign Agricultural Relations. *Foreign Agricultural Trade, United States Foreign Trade in Agricultural Products, Fiscal Year 1945-46*, p. 17. Processed.

Fig. 12.: Same as figure 11, except:

Pork and pork products:

- 1895-1899: Richards, Preston. *Trends in Production and Foreign Trade for Meats and Livestock in the United States*, p. 52. Washington, Gov't Print. Off., (U. S. Dept. Agr. Tech. Bul. 764).
- 1900-1909: U. S. Department of Agriculture. *Yearbook, 1923*, p. 1113.
- 1910-1914: U. S. Department of Agriculture. *Yearbook, 1927*, p. 1107.
- 1915-1939: U. S. Department of Agriculture. *Agricultural Statistics, 1942*, p. 543.
- 1940-1944: U. S. Office of Foreign Agricultural Relations. *Foreign Agricultural Trade, United States Foreign Trade in Agricultural Products*, Apr., 1946, p. 7-10. Processed.
- 1945: U. S. Office of Foreign Agricultural Relations. *Foreign Agricultural Trade, United States Foreign Trade in Agricultural Products, Fiscal Year 1945-46*, p. 17. Processed.

Fig. 13.: Same as figure 11.

Fig. 14.: Same as figure 11 and the following:

- 1910-1934: U. S. Department of Agriculture. *Agricultural Statistics, 1937*, p. 366.
- 1935-1944: U. S. Department of Agriculture. *Agricultural Statistics, 1946*, p. 439.
- 1945: U. S. Office of Foreign Agricultural Relations. *Foreign Agricultural Trade, United States Foreign Trade in Agricultural Products, 1945-46*, p. 8. Processed.

Fig. 15.: Same as figures 1, 5, and 11, and the following:

- 1910-1945: U. S. Bureau of Agricultural Economics. *Agricultural Statistics, 1945*, p. 441.

1946: U. S. Bureau of the Census. *Urban and Rural Population of the United States, by Age and Sex: 1946, 1945, and 1940*, p. 3. Washington, D.C., 1947. Processed. (P-S, No. 19.)

Fig. 16.: U. S. Bureau of Agricultural Economics. *Agricultural Outlook Charts, 1947*, p. 85, 94, and 103. Washington, D.C., 1946. Processed. And: U. S. Bureau of Agricultural Economics. *Consumption of Agricultural Products*, by Elna Anderson, p. 10. Washington, D.C., 1941. Processed. And: U. S. Agricultural Marketing Service. *Farm Production, Disposition, and Income from Milk, 1924-1940, by States*, p. 28. Washington, D.C., 1941. Processed.

Fig. 17.: Prices received by farmers: Same as figure 5.

Income of industrial workers:

1910-1945: U. S. Bureau of Agricultural Economics. *Agricultural Outlook Charts, 1946*, p. 2. Washington, D.C., 1945. Processed.

1946-1947: U. S. Bureau of Agricultural Economics. *Agricultural Situation*, vol. 31, no. 9, p. 14. Sept., 1947.

Income of nonfarm population:

1910-1928: U. S. Department of Agriculture. *Agricultural Statistics, 1942*, p. 663.

1929-1941: U. S. Department of Agriculture. *Agricultural Statistics, 1945*, p. 441.

1942-1946: U. S. Bureau of Agricultural Economics. *Farm Income Situation, FIS-89, June-July, 1947*, p. 21. Processed.

1943-1946: U. S. Department of Commerce. *Survey of Current Business*, vol. 24, no. 4; vol. 27, no. 5, Apr., 1944-May, 1947.

1947: Estimated by obtaining the ratio between U. S. Department of Agriculture figures and the U. S. Department of Commerce figures for the years 1943-1946.

Fig. 18.:

Old series:

1910-1944: U. S. Bureau of Foreign and Domestic Commerce. *Gross National Product or Expenditure, 1909-1944*, 1 p. Washington, D.C., 1946. Processed.

1945-1946: U. S. Board of Governors of the Federal Reserve System. *Federal Reserve Bulletin*, vol. 33, no. 2, p. 206. Feb., 1947.

New series:

1929-1946: U. S. Board of Governors of the Federal Reserve System. *Federal Reserve Bulletin*, vol. 33, no. 9, p. 1109. Sept., 1947.

Fig. 19.:

Old series:

1910-1944: U. S. Bureau of Foreign and Domestic Commerce. *Gross National Product or Expenditure, 1909-1944*, 1 p. Washington, D. C., 1946. Processed. And: Government Statistics Bureau. *Handbook of Basic Economic Statistics*. 1947 Edition, p. 4-5. Washington, D.C., 1947. Processed.

1945-1946: U. S. Board of Governors of the Federal Reserve System. *Federal Reserve Bulletin*, vol. 33, no. 2, p. 206. Feb., 1947.

New series:

1929-1946: U. S. Bureau of Foreign and Domestic Commerce. *National Income*, Supplement to Survey of Current Business, p. 19. July, 1947. Second printing. Washington, Gov't Print. Off., 1947.

1947: U. S. Board of Governors of the Federal Reserve System. *Federal Reserve Bulletin*, vol. 33, no. 9, p. 1167, 1168. Sept., 1947.

The old and new series were linked on the basis of 1929-1939.

Fig. 20.: Same as figures 5, 17, and 19.

Fig. 21.:

Durable manufactures:

1919-1945: U. S. Office of Price Administration. *OPA Handbook of Basic Economic Data*. 2d ed., p. 72-73. Washington, D.C., 1946. Processed.

1946: Computed from an average of monthly figures in Government Statistics Bureau. *Handbook of Basic Economic Statistics*. 1947 Edition, p. 78, 79. Washington, D.C., 1947. And its *Monthly Supplement*, no. 2, p. 13. Feb., 1947. Processed.

Farm machinery:

1910-1945: U. S. Bureau of Agricultural Economics. *Agricultural Prices*, p. 33-34. Apr. 29, 1946. Processed.

1946: U. S. Bureau of Agricultural Economics. *Farm Cost Situation*, FCS-2, p. 1. Mar., 1947. Processed.

Fig. 22.:

Interest rate of prime commercial paper:

1910-1941: U. S. Board of Governors of the Federal Reserve System. *Banking and Monetary Statistics*, p. 448. Washington, D.C., 1943.

1942-1943: U. S. Board of Governors of the Federal Reserve System. *Federal Reserve Bulletin*, vol. 30, no. 12, p. 1215. Dec., 1944.

1944-1946: U. S. Board of Governors of the Federal Reserve System. *Federal Reserve Bulletin*, vol. 32, no. 8, p. 901. Aug., 1946. The 1946 figure is preliminary, based on monthly figures from January to December, 1946.

Yield of corporate bonds: Standard and Poor's Corporation. *Standard and Poor's Trade and Securities Statistics, Long-term Security Price Index Record through December 31, 1940*.

1910-1939: vol. 96, no. 9, Sec. 2, p. 127. Sept., 1941. Standard and Poor's Corporation. *Standard and Poor's Trade and Securities Statistics, Current Statistics Combined with Basic Statistics*.

1940-1941: vol. 97, no. 1, Sec. 2, p. 8. Jan., 1942.

1942: vol. 97, no. 12, Sec. 1, p. 27. Dec., 1942. And: vol. 98, no. 1, Sec. 1, p. 27. Jan., 1943.

1943: vol. 99, no. 1, Sec. 1, p. 27. Jan., 1944.

1944: vol. 100, no. 1, Sec. 1, p. 27. Jan., 1945.

1945: vol. 12, no. 1, Sec. 1, p. 27. Jan., 1946.

1946: vol. 12, no. 8, Sec. 1, p. 27. Aug., 1946. The 1946 figure was obtained from Standard and Poor's *Current Statistics* and compared with *Current Statistics Combined with Basic Statistics*, vol. 31, no. 1, Sec. 1, p. 27. Jan., 1947.

Fig. 23.:

Investment: Same as figure 19.

Quantity of money:

1910-1936: U. S. Board of Governors of the Federal Reserve System. *Banking and Monetary Statistics*, p. 34-35. Washington, D.C., 1943.

1937-1945: U. S. Board of Governors of the Federal Reserve System. *Federal Reserve Bulletin*, vol. 32, no. 8, p. 891. Aug., 1946.

1946-1947: U. S. Board of Governors of the Federal Reserve System. *Federal Reserve Bulletin*, vol. 33, no. 9, p. 1131. Sept., 1947.

Tabulation, page 9: Same as figure 1.

Tabulations, pages 15, 16: U. S. Bureau of Agricultural Economics. *Cash Receipts from Farming by States and Commodities, Calendar Years 1924-44*, p. 154, 158. Washington, D.C., 1946. Processed.

Tabulation, page 17:

Cash income from dry edible beans, potatoes, sweet potatoes, and total truck crops—same as above.

Total truck crops include the following: lettuce, cantaloupes, asparagus, tomatoes, green peas, celery, onions, cauliflower, artichokes, snap beans, watermelons, cabbage, cucumbers, lima beans, beets, carrots, sweet corn, eggplant, escarole, Honey Ball and Honey Dew melons, kale, green peppers, pimientos, and spinach.

Cash income from individual truck crops was computed in the following way: the total farm value for each truck crop was obtained from: U. S. Bureau of Agricultural

Economics. *Estimates of Acreage, Production and Value, 1918-1927 Under New Seasonal Groupings*. Washington, D.C., 1944. Various paging. Processed. *And: Revised Estimates of Acreage, Production and Value, 1928-1941, Under New Seasonal Groupings*. Washington, D.C., 1943. Various paging. Processed. *And: Commercial Truck Crops for Processing [1918-1927]*. Washington, D.C., 1945-1946. Various paging. Processed. *And: Truck Crops for Commercial Processing; Acreage, Production, Price and Value, 1928-1941*. Washington, D.C., 1944. 64 p. Processed. These values were adjusted on the basis of the ratios between cash farm income from truck crops and farm value of truck crops.

Tabulation, page 23: U. S. Bureau of Agricultural Economics. *Income Parity for Agriculture. Part VI—State Estimates of Income and Production Expenses. Section 1. Net Income and Production Expenses of Farm Operators by States, Calendar Years 1929, 1939-44*, p. 40-51. Washington, D.C., 1945. Processed.

Tabulation, page 24:

Cash farm income: Same as figure 1.

Value of domestic agricultural exports:

1910-1914: U. S. Department of Agriculture. *Agricultural Statistics, 1940*, p. 485.

Converted from a July-June basis to a calendar year basis by straight-line interpolation.

1915-1944: U. S. Office of Foreign Agricultural Relations. *Foreign Crops and Markets, Calendar Year Supplement*, Dec. 1, 1945, p. 3. Processed.

1945: U. S. Office of Foreign Agricultural Relations. *Foreign Agricultural Trade, United States Foreign Trade in Agricultural Products*, Mar., 1946, p. 6. Processed.

H I L G A R D I A

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HERBICIDAL PROPERTIES OF OILS^{1, 2}

A. S. CRAFTS³ and H. G. REIBER⁴

INTRODUCTION

Oils as Herbicides. Oils of many types have been employed as herbicides. In the West, petroleum fractions have been most popular; and of these Diesel oil, stove oil, and smudge-pot oil have been applied in greatest volume. They have been used to kill general weed growth on roadsides and railway roadbeds in order to prevent the spread of fires. They are a means of eliminating the vegetation that depletes soil-moisture reserves in citrus groves; of destroying the weeds that harbor insect and fungus pests on ditchbanks, fence rows, and similar untilled areas; and of burning undesirable growth on irrigation ditches. Over 3 million gallons have been consumed annually in California for these purposes, and the use is expanding rapidly. Recently stove oil has been applied as a selective spray against weeds in vegetable crops, notably carrots, celery, and parsley.

The herbicidal use of oils has developed through many years. Early practices utilized acid sludge, acid tar, Edeleanu extracts, and other by-products of oil refining that had little commercial value. In the East, residues from coal and wood distillation, various petroleum fractions, gas oils, and similar low-cost products have been applied to weeds. Availability and hauling cost have largely determined distribution and the extent of use.

Increased demand for oils as motor and heating fuel has been accompanied by improvement in the quality of these products; herbicidal toxicity has likewise been altered. Unsaturated compounds, particularly the aromatics, tend to make fuel oils burn sooty, and they have antiknock properties that are undesirable in a Diesel fuel. These compounds are toxic to plants, and their removal decreases the killing action of an oil. One problem is that of providing enough toxicity per unit volume to kill plants with the amount required to wet them.

Specificity of Oils. Weed species vary in their response to oil injury. Members of the Umbelliferae are notably tolerant of oils; and the crop plants

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² Many of the samples used in these studies were provided by the Shell Development Company, the Tidewater Associated Oil Company, the Dow Chemical Company, and Standard Agricultural Chemicals, Inc. The writers wish to express their appreciation for this cooperation. We are also indebted to Mr. H. W. Allinger, Principal Laboratory Technician, Division of Chemistry, for most of the analytical work reported in this paper.

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carrot, celery, parsnip, parsley, and anise are so resistant that stove oil is used as a selective spray to kill weeds in them. Even these crops are injured by oils heavier than stove oil. Toxicity also varies with temperature, being higher during hot sunny weather than on cool days.

Oil residues are distasteful on crops. If a selective stove-oil spray has been applied late in the growing season, there may be insufficient time for the residue to be dissipated and the product may be unacceptable on the market. On carrots the stove-oil spray must be applied while the seedlings have one to four true leaves; later applications leave an oily residue undesirable in the cooked vegetable. The question is often asked: Can the manufacturer produce an oil that will be nontoxic to carrots and will leave no objectionable residue? Another common inquiry is whether oils might serve as selective herbicides on other crops.

Fortified Oils. Research workers on herbicides have discovered several chemicals that will make oils more toxic and will broaden their applicability to oil-tolerant weed species. Since the use of such "fortifying" agents is relatively new, information is needed concerning the type most desirable for various weed populations, the quantities most economical to use, the kind of oil best suited to combine with them, and the proper amendments required to render the herbicidal mixture most compatible with the methods of application.

New Types of Oils. Finally, new oil fractions and even new oils are being made available for weed control. The users need help in fitting these new products into the general schemes of weed control employed in the regions where each product will be marketed.

Scope and Purpose of This Study. As this brief introduction shows, there are many uses for oils in weed control, and oil products fulfill needs that other chemicals cannot satisfy. For efficiency, however, the properties of these oils must be understood; only thorough tests can clarify the relation between toxicity and chemical composition, particularly with respect to selective action on different plant species.

The research described in this paper is a preliminary survey and does not attempt a detailed treatment subject to statistical analysis. Presentation of data has been organized on a topic basis to center around oil types rather than crops, weeds, or weed-control methods. Later papers, of a more popular type, will describe the more practical aspects of the use of oils in weed control. These will stress crops, weeds, and control methods.

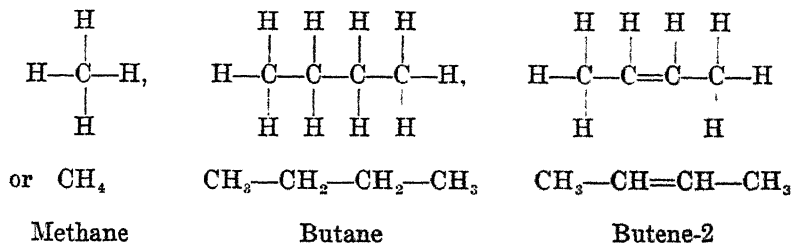
COMPOSITION OF OILS

Classification of Oils. Oils may be classified in many ways. Naturally occurring oils of prehistoric origin are petroleum, shale oil, and gas-drip oil. These may find their way into the herbicide field in practically their natural condition; various fractions are commonly used; and altered oils (produced by destructive distillation, cracking, or catalytic synthesis) may be employed.

Petroleum is largely composed of the chemical compounds collectively named hydrocarbons, together with a few other organic compounds containing oxygen, sulfur, and nitrogen. Refined petroleum products, isolated mainly on the basis of boiling range, are available commercially and are widely used.

The less refined of these are stove oil, Diesel fuel, and smudge-pot oil; and the more refined are gasoline, kerosene, cleaner's naphtha, commercial solvents, and lubricating oils. This report considers some chemical properties of those types of compounds that are found in petroleum or produced from it. Their chemical structure is briefly described for those unfamiliar with oil chemistry.

Hydrocarbons are compounds of carbon and hydrogen, combined, in their simpler forms, as indicated by the following formulas:



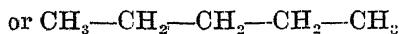
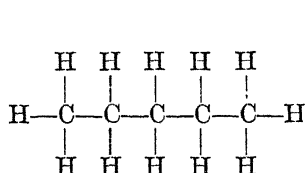
The carbon and hydrogen atoms are joined by one or more chemical bonds (each indicated by a dash), dispersed in conformity with the tetravalent character of carbon. Although the examples given above are simple in structure, the carbon chain could be extended to many atoms; side chains of carbon atoms could be attached at many points; the chain could be formulated into ring structures (usually limited to 5 or 6 carbon atoms); and double or even triple bonds might be present in many positions.

Since the number of possible hydrocarbons is extremely large, the need for classification seems obvious. Actually, it has proved difficult to isolate, from petroleum, individual hydrocarbons of more than 6 carbon atoms. The explanation is that with increasing chain length of the compound, the number of possible atomic arrangements increases tremendously, but the difference between boiling points rapidly decreases. Boiling range, therefore, is widely used for an approximate characterization of petroleum fractions, whereas chemical criteria are applied whenever a more exact classification is desired.

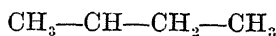
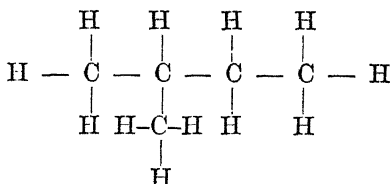
Saturated Hydrocarbons. Most of the hydrocarbons in petroleum belong to the saturated paraffins, so called because their carbon atoms are singly bonded. These have the general formula $\text{C}_n \text{H}_{2n+2}$, where the subscript designates the number of atoms per molecule, and n is any integer. Two of the simpler members of the series—methane, CH_4 , and butane, $\text{C}_4 \text{H}_{10}$ —are shown above. Separation of these saturated hydrocarbons on the basis of boiling range is common. Compounds containing 1 to 4 carbon atoms make up the gaseous hydrocarbons and are the main constituents of natural gas. The 5- to 7-carbon hydrocarbons have boiling points varying from 70° to 210° F approximately and are marketed under the name of ligroin or petroleum ether. Succeeding groups include (1) the natural, straight-run gasoline fraction, compounds having 6 or 7 to 12 carbon atoms and a boiling range from 110° to 390° F; (2) the kerosene fraction, having 12 to 15 carbon atoms and boiling from 390° to 525° F; (3) the gas oil fraction having 15 to 18 carbons and boiling from 480° to 570° F. Stove oil has a boiling range of 330° to 570° F; Diesel

oil, 400° to 700° F. Lubricating oil, greases, vaseline, and paraffin include those compounds having 16 to 24 carbon atoms; their distillation residue is mainly a petroleum coke or asphalt tar. The lower-boiling fractions of petroleum—for example, gasoline or kerosene—are often called light oils, as compared with the higher-boiling fractions termed heavy oils. Since the basis for the above fractionation is boiling range, one can enlarge any fraction considerably by varying the limits; the figures above are approximations only. Ordinary refinery procedures for preparing such paraffinic fractions are well known, and the products are available. Further fractionation of these products made it possible to study the relation between herbicidal properties and the boiling range of petroleum hydrocarbons.

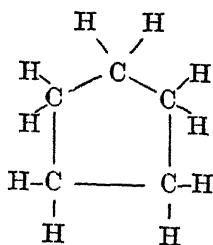
The saturated hydrocarbons $C_n H_{2n+2}$ have been classified into three main groups: the normal or straight-chain compounds, illustrated by pentane; the branched-chain compounds such as isopentane; and the ring structures such as cyclopentane. Typical formulas are as follows:



Pentane



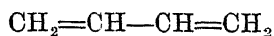
Isopentane



Cyclopentane

The ring structures, including the 6-carbon-ring compound cyclohexane, with and without side chains, are termed naphthenes in petroleum technology. The saturated hydrocarbons are, with a few exceptions, rather inactive chemically; and they are the main ingredients of highly refined petroleum products such as gasoline, kerosene, and lubricating oils.

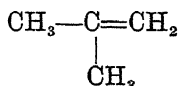
Unsaturated Hydrocarbons. Those hydrocarbons containing carbon-to-carbon double or triple bonds are generally termed olefinic or unsaturated compounds. Typical olefins of low boiling point are the following :



Butadiene



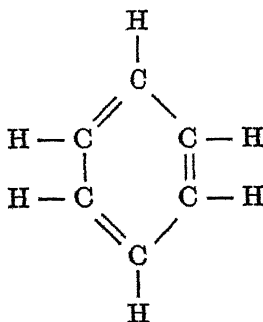
Heptene-1



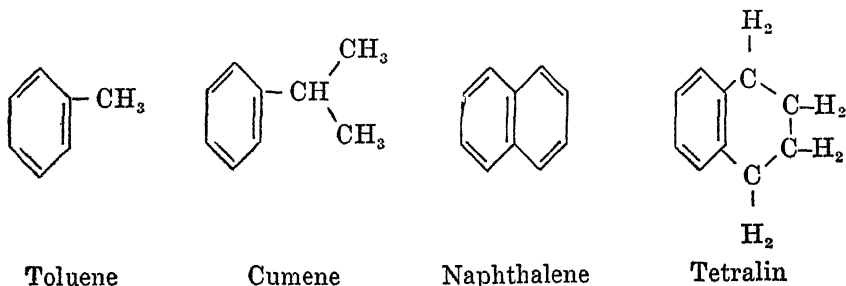
Isobutylene

These are in general more reactive than the saturated hydrocarbons; they may be removed from petroleum fractions by treatment with concentrated sulfuric acid, a common procedure in oil refining. The unsaturated hydrocarbons in refined petroleum assumed great importance when the cracking process was introduced. By this process large, relatively nonvolatile hydrocarbon molecules are broken down thermally into smaller molecules of the volatility desired in gasoline. The amount of this fuel obtained from petroleum was thereby practically doubled. The quantities of olefins formed during cracking are considerable, and are now known to increase the octane rating of gasoline. The additional refinery processes called dehydrogenation, polymerization, and alkylation, made possible by the reactivity of olefins or alkenes, have proved valuable in the synthesis of high-octane gasolines. Hence unsaturated compounds play an important role in modern refinery procedures, and they may be found in varying amounts in different products.

Aromatic Hydrocarbons. One type of unsaturated compound differing materially, in chemical properties, from those above forms an entire series, known as the aromatics. These are generally considered as derivatives of benzene. The term *aromatic* was originally applied because the earliest-known compounds of this type, such as cinnamon and oil of wintergreen, have agreeable odors. The structure of benzene, C_6H_6 , is commonly represented as a 6-carbon cyclic structure with alternate single and double bonds.



The resonance theory, developed more recently, proposes that the bonds are actually all alike; but some degree of unsaturation is indicated by their limited reactivity with certain reagents. When the hydrogen atoms of the benzene ring are substituted by carbon chains or by other cyclic structures, higher-boiling aromatic compounds naturally result. A number of such compounds from gasoline and a number of naphthalenes from kerosene have been isolated. A few typical compounds are shown.



Other Compounds in Oils. Sulfur compounds occur in petroleum, usually to the extent of less than 1 per cent. They are of the paraffin type—for example, mercaptans, pentamethylene sulfides—or unsaturated ring structures such as thiophene. As a rule, these compounds are objectionable in oils. Common refinery procedures eliminate them almost completely.

Nitrogen compounds of the quinoline type are present in petroleum to less than 0.1 per cent. These nitrogen bases are readily removed by acid extraction.

Oxygen compounds (0.1 to 3 per cent) are usually acidic and are removed by alkaline washes. They are in general either phenols or cycloparaffinic carboxylic acids, the latter being termed, in petroleum technology, naphthenic acids. Many refined petroleum fractions, upon standing in the presence of oxygen, are subject to oxidation, with the production of peroxides and acids. Such reactions often cause the deterioration of fuel and lubrication oils, and they are commonly inhibited by the presence of naturally occurring or added antioxidants. The herbicidal properties of petroleum fractions may be appreciably altered by such oxidation, as will be reported in this paper.

Oil Refining. Refinery procedure for the treatment of petroleum has undergone and is still undergoing rapid development. From simple distillation followed by chemical treatment, refinery procedures have advanced to include cracking, re-forming, solvent extraction, isomerization, polymerization, alkylation, hydrogenation, and dehydrogenation. These processes, including both destructive fragmentation and constructive synthesis, constitute a complex field of chemical technology that makes almost innumerable products available for testing. Such products, donated by various oil companies interested in weed control, have contributed much to the studies reported here.

Besides petroleum fractions, a few additional oils may be mentioned. Gas-drip oil, a condensation product from natural gas, has been found low in herbicidal toxicity. Small lots might be fortified with dinitro compounds and made toxic enough for herbicidal purposes where other uses cannot be found.

Nonpetroleum Oils. Oils recovered from the distillation of coal are highly aromatic and have proved very toxic to all plants. Since, however, they contain valuable compounds recoverable for solvents and organic syntheses, only small quantities are available for killing weeds. Ammonium thiocyanate, a chemical obtained from coal distillates by washing, has been tried for weed control; but it failed in competition with sodium chlorate. Still residues containing crude cresylic acid and similar residues have found local use; but most of this material has been needed for the preservation of timbers, posts, and ties.

As with coal, some oils from the destructive distillation of woods have been used for weed control. These have been mostly still residues from which the easily recoverable materials have been taken. One such product, a crude residue high in cresylic acid, proved extremely toxic to all plants. Since, however, it could not be easily diluted, it served only as a straight oil spray and, because of the hauling cost, could be used only near the point of production.

The popularity of fuel oils for weed control in the West is mostly due to their large-scale production and low cost. Whenever oil prices rise sufficiently, other materials (for example, chlorates, thiocyanates, sulfamates, and dinitro-substituted phenols) become more economical for general weed control. The use of oils as selective herbicides is a different matter. For this purpose, in certain vegetable crops, there seems at present to be no substitute for light petroleum fractions. After sufficient refinement, this method of attacking weeds may well become standard practice, eliminating costly hand labor and providing a higher degree of control over all operations involved.

TOXICITY OF OILS

Though the toxicity of oils to plant foliage has long been recognized, the exact chemical mechanism is not clearly defined. Innumerable observations have been made by commercial sprayers, and for years it has been known that the crude fractions of petroleum obtained by distillation are more toxic than refined oils. Acid treatment that removed unsaturates and aromatics reduced the toxicity, and the relatively nontoxic oils used as dormant sprays on deciduous trees and as scale sprays on citrus are produced by heavy acid refining or solvent extraction. Such treatment with concentrated sulfuric acid enables one to remove the olefins and aromatics, leaving only the aliphatic and naphthene compounds. Thus Gray and de Ong (1926)⁵ found the sulfonation test to be the most reliable laboratory index of toxicity; they observed that oils having an unsulfonated residue of only 50 to 60 per cent were exceedingly toxic, whereas those with an unsulfonated residue of 90 per cent or above were relatively nontoxic.

That the aromatic compounds are highly toxic has been recognized. The Tidewater and Associated Oil Company has sold a herbicidal oil high in aromatics under the name of Avon Weed Killer. This product, a residue in the refining of kerosene by the Edeleanu process, contains a large proportion of high-boiling aromatic compounds. It is extremely toxic.

That different fractions have different herbicidal properties is evident from the recommendations for their use. For instance, gasoline has been recom-

⁵ See "Literature Cited" for citations, referred to in the text by author and date.

mended for spot treatment of individual dandelion plants in lawns, whereas kerosene containing not over 4 per cent unsaturates is preferred as a selective spray for dandelions. If gasoline or any fraction higher than 4 per cent in unsaturates is used as a spray, the grass is injured.

Toxicity Types. Two types of toxicity were noted on citrus by de Ong, Knight, and Chamberlin (1927) : acute toxicity, a rapid burning of leaves by oils of low boiling point; and chronic toxicity, a slow yellowing of leaves by oils high in boiling range but fluid enough to be applied by spraying. In the herbicidal use of oils similar toxicity types have been observed. Acute toxicity, characteristic of light oils, results in rapid burning of leaves and stems. If injury is not complete within 24 to 48 hours, the plants may recover by growth of axillary buds or, with grasses, by tillering.

Chronic toxicity commonly results from oils heavy enough to give an oil-soaked appearance, and persistent enough to remain visible on the plants for several days or more. Injury symptoms of chronic toxicity usually develop slowly and may appear only after several days. They consist of a yellow blotching of leaves with slow death. Also in evidence is killing of the inner leaves of grasses. Growth is always retarded, and often secondary infection by fungi is prominent.

Killing by gasoline, engine distillate, and stove oil is largely acute; that by Diesel oil, light furnace fuel, kerosene, and light lubricating oils is chronic. The extent of injury and rapidity of killing by all oils depends upon the amount of refinement they have undergone; highly refined oils that have been largely freed of unsaturated compounds are less destructive than unrefined fractions. For further discussion of acute and chronic toxicity see page 147.

Selectivity. Another aspect of oil toxicity is the selective action on certain plant species. As noted above, the family Umbelliferae tolerates oils; and stove oil will kill weeds with little injury to carrots, celery, parsnips, parsley, and the like. If Diesel oil is used, however, the leaves burn slowly. Evidently these crop plants can endure the acute type of injury, but chronic toxicity affects them as it does the weed species. As a trial in the field has shown, third-structure gasoline is even more toxic to weeds than is stove oil; it has no toxicity for carrots, and it evaporates more quickly, leaving less distasteful residue.⁶ When allowed to stand in the light, however, it becomes toxic and loses selectivity.

Different weed species vary in their response to oil sprays. Wild mustard, *Amsinckia species*, and the pigweeds are susceptible to oil toxicity. *Chenopodium murale* is more easily killed than *C. album*. Common milkweed and wild lettuce are intermediate in susceptibility. Chickweed is not only somewhat tolerant to oil, but difficult to cover thoroughly. Annual nettle is also hard to kill completely.

Malva, yellow star thistle, groundsel, pineapple weed, and purslane resist stove oil and even tolerate Diesel oil. Umbelliferae such as fennel, poison hemlock, and wild carrot are also highly tolerant.

⁶ Since this manuscript was completed, two large oil companies have placed on the market special selective spray oils for weed control in carrots and related crops. These are lighter oils of narrower boiling range than stove oil. They have the advantage of gasoline and lack its hazards.

LABORATORY AND GREENHOUSE METHODS

With the foregoing observational knowledge as a background and with many questions on the nature of oil toxicity as a stimulus, experiments were initiated aiming, first, to elucidate the chemical mechanics of the response of plants to oil sprays; second, to find new and better oil products for both selective and general weed spraying. Greenhouse plants growing in 12-inch pots and in no. 10 cans were used for toxicity tests, spraying being accomplished with a sprayer⁷ while the plants in a pot or can are rotated on a motor-driven turntable.

After being sprayed, the cultures were returned to the greenhouse benches. Readings were made periodically to determine the extent of injury. Acute injury was usually apparent within a few hours; within 24 hours after spraying estimates of injury were made in order to measure the inception of this type of toxicity.

The early experiments were observed for relatively short periods, usually a week or less, because the full significance of chronic injury was not appreciated. Later the periods of observation were lengthened; some extended for a month or more; chronic injury had usually run its course within 6 weeks.

All data on injury were visual estimates of the amount of damage caused by the sprays, as compared with unsprayed controls. Estimates of acute toxicity are quite accurate, particularly in the low and high ranges. Chronic injury in the high range can be accurately estimated; in the low range plants are often retarded in growth with little visible killing of tissue. This effect is difficult to evaluate, and probably all estimates on this type of damage are low. Such retardation of growth is of little importance where complete control by a general contact spray is sought; in selective spraying, however, it may be highly important; for it allows the crop plants to outgrow and shade weeds to such an extent that the weeds offer little competition. This would be particularly true of wild oats in a flax crop.

Estimates of 100 per cent toxicity indicate that all foliage has been killed. When later readings drop in value, regrowth from axillary buds has taken place. Injury of 100 per cent on annual grasses usually indicates complete killing. On *Chenopodium* species, carrots, and some other plants, sprouting from axillary buds commonly occurred.

In the early experiments mixed cultures of plants were used, often consisting of one crop plant plus one or more weed species. Later it proved more satisfactory to grow pure plant cultures and to use two or more species in each test, the particular species being determined by the nature of the test.

Other problems were encountered: the need for a nontoxic diluent to use in varying the dosage of the more toxic materials; the question of volume dosage as related to size of plant and extent of wetting; the proper age and development of the different plants, particularly with respect to selective action; and the ever-present difficulties of controlling light, temperature, humidity, and so forth, under greenhouse conditions.

The problem of a nontoxic diluent has not been solved. Odorless kerosene, a heavy isoparaffin, and normal cetane have been tried; all were nontoxic on

⁷ The De Vilbiss atomizer no. 261 has proved suitable.

carrots, but even the cetane produced physiological disturbances on barley. Volume dosage was standardized by trial and error on plants of different sizes and, unless otherwise noted, consisted of the amount required to thoroughly wet the foliage of the plants. Variability in such factors as plant age, light, temperature, and humidity was largely eliminated by growing enough plants so that a complete set of oil fractions could be tested simultaneously. Since there was no possibility of accurately controlling the greenhouse environment, experiments conducted at different times of the year are not strictly comparable, and some erratic results undoubtedly were due to such differences, particularly in temperature.

OIL FRACTIONS

The testing of the herbicidal properties of oil fractions developed from some practical problems posed by carrot growers who were using stove oil as a selective spray. They asked, "Is stove oil the best fraction to use for carrot

TABLE 1
SPECIFICATIONS* ON STOVE-OIL SAMPLES

Source of oil	Initial b.p., ° F.	Temperature, ° F, at			End point, ° F.	Grav- ity, ° A.P.I.	Vis- cosity, Say- bolt, sec.	Flash point, P.M. c.c., ° F.	Pour point, ° F.	Sulfur, per cent	Ash, per cent	* B.S.W. †
		10 per cent recov- ery	50 per cent recov- ery	90 per cent recov- ery								
Company A.....	340- 382	552- 558	37.6- 38.5	31- 33	134- 140	0-20	0.13- 0.68
Company B†.....	345	390	445	500	572	38.5	32.5	136	-25	0.18	0.01	0
Company C..	390	480	38.6	138	Below 0	0.21
Company D§.....	335	390	...	515	565	38.7	32	134	Below 0	Below 0.5	Trace	Trace

* These specifications were supplied by the companies providing the samples.

† Sediment and water.

‡ Color 1+; color not stated for other oils.

§ Ignition quality 51 Cetane; not stated for other oils.

TABLE 2
TOXICITY OF GASOLINE TO *Amsinckia*, *Lactuca*, *Poa annua*, AND BARLEY,
IN CARROT CULTURES;* DECEMBER 15, 1943†

Amount of gasoline applied per culture	Injury to <i>Amsinckia</i> (A), <i>Lactuca</i> (L), <i>Poa annua</i> (P), and barley (B), in per cent															
	1 day				2 days				3 days				5 days			
	A	L	P	B	A	L	P	B	A	L	P	B	A	L	P	B
3 ml.....	50	20	80	15	60	40	75	20	75	60	75	20	90	75	100	25
6 ml.....	75	40	70	60	85	50	90	60	90	50	90	75	95	90	100	90
9 ml.....	95	75	50	90	100	95	95	95	100	100	95	100	100	100	100	100
12 ml.....	100	80	75	95	100	95	95	95	100	100	100	100	100	100	100	100
15 ml.....	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

* No injury to carrots was evident at volume rates as high as 24 ml per culture.

† Date of application.

TABLE 3
TOXICITY OF STOVE OIL TO CARROTS, WEEDS,* AND GRASS;
JANUARY 30, 1943

Amount of stove oil applied per culture	Injury to carrots (C), weeds (W), and grass (G), in per cent								
	1 day			3 days			6 days		
	C	W	G	C	W	G	C	W	G
1 ml.	0	10	0	0	15	10	0	20	10
2 ml.	0	10	50	0	15	50	0	20	50
3 ml.	0	75	90	0	75	90	0	75	90
4 ml.	0	90	90	0	90	95	0	90	95
5 ml.	5	90	90	5	90	95	5	95	100
6 ml.	5	90	90	10	95	95	10	95	100

* The weeds referred to here were *Brassica*, *Amsinckia*, and *Lactuca* species.

TABLE 4
TOXICITY OF GASOLINE, STOVE OIL, AND DIESEL OIL TO WEEDS,* GRASS, CARROTS,
AND FLAX; FEBRUARY 28, 1944

Oil and amount applied per culture	Injury to weeds (W), grass (G), carrots (C), and flax (F), in per cent															
	1 day				2 days				3 days				5 days			
	W	G	C	F	W	G	C	F	W	G	C	F	W	G	C	F
Gasoline:																
7.5 ml.	25	50	0	25	45	100	0	50	50	100	0	50	50	100	0	75
15.0 ml.	60	50	0	95	85	100	0	100	85	100	0	100	85	100	0	100
Stove oil:																
7.5 ml.	90	60	0	95	95	95	5	100	97	100	5	100	99	100	10	100
15.0 ml.	90	60	0	95	99	100	15	100	99	100	20	100	100	100	20	100
Diesel oil:																
7.5 ml.	40	40	5	80	60	50	5	100	75	60	5	100	99	75	10	100
15.0 ml.	45	40	5	80	70	50	5	100	85	60	10	100	99	90	15	100
	7 days				11 days				15 days				24 days			
	W	G	C	F	W	G	C	F	W	G	C	F	W	G	C	F
Gasoline:																
7.5 ml.	50	100	0	75	55	100	0	60	55	100	0	50	50	100	0	50
15.0 ml.	87	100	0	100	85	100	0	100	85	100	0	100	85	100	0	100
Stove oil:																
7.5 ml.	100	100	15	100	100	100	15	100	100	100	15	100	100	100	30	100
15.0 ml.	100	100	30	100	100	100	40	100	100	100	40	100	100	100	60	100
Diesel oil:																
7.5 ml.	100	90	10	100	100	100	25	100	100	100	40	100	100	100	90	100
15.0 ml.	99	100	20	100	100	100	60	100	100	100	75	100	100	100	100	100

* Weeds referred to here were *Brassica*, *Amsinckia*, and *Lactuca* species.



A

Fig. 1.—Effects of oil sprays on plant cultures. A, Untreated check; left, carrots; center, barley; and right, flax. B, Similar cultures sprayed with stove oil. The flax and barley were killed; the carrots were not injured.

spraying? Are various brands of stove oil equally effective? How much should be applied? Why does Diesel oil injure carrots?" and many similar questions.

Comparison of Stove Oil, Gasoline, and Diesel Oil. One early experiment compared four stove-oil samples obtained from different companies. Table 1 gives the common specifications on these oils. Tested on cultures of carrots, mustard, fiddleneck, and annual bluegrass, these four samples showed no significant differences. As shown in table 1, the gravity ratings of the samples were almost identical. Recently stove oil with an A.P.I. gravity rating of 34.7° proved toxic to carrots in the field. This fact suggested that an oil only slightly heavier than stove oil might contain compounds injurious to carrots.

In contrast to this result, gasoline proved harmless to carrots but highly toxic to weeds. A sample of white gasoline was used in the tests, and volumes ranging from 3 to 24 ml per culture were applied. Even the highest rate (about four times the usual volume for such cultures) proved nontoxic to the carrots. Table 2 gives the results on barley and on three weed species.

In a volume-rate test of stove oil on carrots, mixed weeds, and grass, when as much as 5 and 6 ml of this material was used per culture, the carrots suffered slightly. Judging from table 3, dosage might be critical in field application of stove oil. This hypothesis is confirmed by reports of occasional injury to carrots when heavy dosages are used in commercial application.

*B*

According to these few experiments, an oil lighter than stove oil might be less hazardous to use, stove oil is just on the margin of safety as a carrot spray, and therefore Diesel oil would be too heavy. Tests confirmed the reported toxicity of Diesel oil to carrots. Table 4 compares gasoline, stove oil, and Diesel oil at two different dosages. Gasoline proved nontoxic to carrots, and at the lower dosage it only partially injured the weeds. Mixed weeds somewhat older than those reported in table 2 were not completely killed at the 15-ml application rate. Figure 1, *A* shows untreated cultures of carrots, barley, and flax; figure 1, *B* shows similar cultures sprayed with stove oil. Figure 2, *A* shows the results of gasoline treatment.

The toxicity of stove oil to carrot cultures developed slowly, but within 24 days damage to the outer leaves was apparent. Later the inner leaves grew, and the carrots recovered. Diesel oil, on the other hand, produced chronic injury that eventually killed the carrots.

Narrow Distillation Fractions. Fractionation of gasoline, stove oil, and Diesel oil, each into four equal-volume cuts, produced samples that showed more definitely the relations between boiling range and toxicity. Table 5 shows the injury to mixed weeds, grass, and carrots at intervals of 2, 4, and 7 days after application. These data prove that injury to carrots is caused by the heavier two fractions of stove oil and by all but the lightest of the Diesel-oil fractions.

These three samples of oil all caused acute toxicity to weeds and grass. On the other hand, only the heavier fractions affected the carrots; and injury



A

Fig. 2.—A, Carrot, barley, and flax cultures sprayed with white gasoline. Injury was very rapid but under the high temperature conditions of the experiment the gasoline evaporated so rapidly that the barley received only a partial contact injury. The flax was killed. B, Similar cultures sprayed with a fresh sample of kerosene. The barley succumbed to the slow chronic injury; carrots and flax were uninjured. Similar results followed spraying with the unsulfonated residue of stove oil.

was chronic. Apparently, acute toxicity results from low-boiling fractions, whereas chronic toxicity is characteristic of the heavier fractions in the Diesel oil and stove oil.

All fractions of the gasoline were highly volatile; the lighter ones evaporated almost as rapidly

as they wet the plants. This volatility determines exposure time. The difference between acute and chronic toxicity may well relate, at least partly, to the difference in time of contact of the toxicants.

Carrots, apparently, tolerate the acute toxicants of oil fractions, but are susceptible to the chronic toxicants of fractions with boiling ranges above 424° F.

Toxicity of Straight-Run and Cracked Gasolines. The results of experiments reported in tables 2 and 4 show that gasoline is toxic to plants and has highly selective properties. Two pertinent questions arise. First, by eliminating the highly inflammable light end of the normal gasoline fraction, can one produce an oil that will surpass stove oil in selectivity and in absence of residue? Second, what is the nature of gasoline toxicity, and what other roles may gasoline play in weed control?

Because these and other questions were frequently recurring, studies on gasoline were made. Table 5 presents data collected over 7 days on the toxicity

**B**

of gasoline fractions to broad-leaved weeds, grass, and carrots. Table 6 gives a more extended view of the toxicity relations of such fractions; the observations were made during a period of 15 days on grass and also on onions, a crop more sensitive than carrots to oil injury. Two applications—12 ml and 6 ml per culture—were made with each fraction. Tests were also made on samples refluxed with and without air; these refluxed samples will be discussed later.

Toxicity to onions by these fractions was not permanent; the plants grew out of the injury and recovered completely by the end of one month. Grass, on the other hand, was severely injured by the lightest fraction and killed by the other three. As in the previous test, toxicity increased with gravity through the series—a fact indicating that the lighter fractions, either because they were high in volatility or because the compounds present were less toxic, caused lighter damage than the heavy ones. Evidently a narrower fraction, excluding the more volatile end, would be more toxic to weeds than the broader cut represented by commercial gasoline. Table 5 shows that the narrower fraction would still be selective enough not to injure carrots. And as field tests on carrots have proved, the residual odor and taste caused by gasoline are less lasting than those of stove oil.

These results indicate that the most convenient and practical method for improving a selective spray oil for carrots would be to use a narrower cut equivalent to approximately the heavy 50 per cent of gasoline or the light 50 per cent of stove oil. Such an oil would have a boiling range of approximately 300° to 425° F.

TABLE 5
TOXICITY OF OIL FRACTIONS TO WEEDS,* GRASS, AND CARROTS;
SEPTEMBER 23, 1943

Oil, fraction no., and boiling range in ° F	Injury to weeds (W), grass (G), and carrots (C), in per cent								
	2 days			4 days			7 days		
	W	G	C	W	G	C	W	G	C
Gasoline:									
No. 1, 100°-227°	60	90	0	70	90	0	75	90	0
No. 2, 227°-293°	90	90	0	99	90	0	99	95	0
No. 3, 293°-338°	95	95	0	100	100	0	100	100	0
No. 4, 338°-420°	100	95	0	100	100	0	100	100	0
Stove oil:									
No. 1, 330°-392°	90	90	0	100	90	0	100	98	0
No. 2, 392°-424°	80	95	0	100	95	0	100	100	0
No. 3, 424°-473°	75	90	0	95	95	5	100	100	10
No. 4, 473°-	60	80	5	90	90	20	100	95	50
Diesel oil:									
No. 1, 392°-487°	75	85	0	95	98	0	100	100	0
No. 2, 487°-545°	70	75	0	95	90	5	100	100	10
No. 3, 545°-590°	60	60	10	90	100	20	100	100	50
No. 4, 590°-	40	35	20	80	100	50	100	100	100

* Weeds referred to here were *Brassica*, *Amsinckia*, and *Lactuca* species.

TABLE 6
TOXICITY OF FRACTIONAL DISTILLATES OF WHITE GASOLINE TO ONIONS AND GRASS;
MAY 5, 1944

White gasoline fraction no. and amounts of gas and diluent (isoparaffin) applied per culture	Injury to onions (O) and grass (G), in per cent									
	1 day		3 days		5 days		10 days		15 days	
	O	G	O	G	O	G	O	G	O	G
Fraction no. 1:										
12 ml gas, 0 ml IP.	20	90	10	90	5	90	0	90	0	95
6 ml gas, 6 ml IP.	0	10	0	10	0	10	0	20	0	25
Fraction no. 2:										
12 ml gas, 0 ml IP.	30	90	20	90	10	95	0	100	0	100
6 ml gas, 6 ml IP.	0	10	0	15	0	15	0	25	0	40
Fraction no. 3:										
12 ml gas, 0 ml IP.	50	95	50	100	40	100	40	100	40	100
6 ml gas, 6 ml IP.	10	50	10	60	5	75	0	90	0	90
Fraction no. 4:										
12 ml gas, 0 ml IP.	65	95	60	100	50	100	30	100	20	100
6 ml gas, 6 ml IP.	15	75	15	85	10	90	0	90	0	90
Fraction no. 3, refluxed without air:										
12 ml gas, 0 ml IP.	50	95	50	95	50	95	40	100	40	100
6 ml gas, 6 ml IP.	0	40	0	40	0	40	0	50	0	50
Fraction no. 3, refluxed with air:										
12 ml gas, 0 ml IP.	50	90	30	90	20	95	0	100	0	100
6 ml gas, 6 ml IP.	0	25	0	30	0	50	0	60	0	90
Fraction no. 4, refluxed without air:										
12 ml gas, 0 ml IP.	50	90	40	90	30	95	20	100	10	100
6 ml gas, 6 ml IP.	10	40	10	40	0	50	0	50	0	75
Fraction no. 4, refluxed with air:										
12 ml gas, 0 ml IP.	70	90	60	90	50	95	40	100	40	100
6 ml gas, 6 ml IP.	0	75	0	75	0	75	0	75	0	90

Because most gasolines contain hydrocarbons produced by cracking, it seems desirable to compare the toxicities of cracked and straight-run samples. Two fresh samples received in April, 1944, had the following properties:

	Straight-run	Cracked
Boiling points of different fractions:		
Initial boiling point.....	100° F	150° F
10 per cent.....	170°	180°
20 per cent.....	177°	195°
30 per cent.....	181°	198°
40 per cent.....	185°	203°
50 per cent.....	189°	206°
60 per cent.....	192°	209°
70 per cent.....	196°	212°
80 per cent.....	200°	215°
90 per cent.....	206°	218°
97 per cent.....	215°	228°
Bromine number	0.67	38.85
Per cent unsaturated.....	0.60 per cent	37.60 per cent
Unulfonated residue	78.2 per cent	59.9 per cent

According to these data, the cracked gasoline has a much higher degree of unsaturation and therefore should be more toxic (see p. 108 to 112). Table 7 shows the results of a preliminary test on wild mustard, run at three concentrations.

TABLE 7
TOXICITY OF STRAIGHT-RUN AND CRACKED GASOLINE ON *Brassica*;
MAY 5, 1944

Type of gasoline and amounts of gas and diluent (isoparaffin) applied per culture	Injury to <i>Brassica</i> , in per cent			
	1 day	2 days	4 days	7 days
Straight-run gasoline:				
10 ml gas, 0 ml IP.....	85	90	90	90
7½ ml gas, 2½ ml IP.....	20	20	20	20
5 ml gas, 5 ml IP.....	0	0	0	5
Cracked gasoline:				
10 ml gas, 0 ml IP.....	95	95	95	100
7½ ml gas, 2½ ml IP.....	75	85	90	90
5 ml gas, 5 ml IP.....	20	20	30	60

These results confirm the prediction that the cracked gasoline would be more toxic than the straight-run sample. The shift in toxicity with time is also greater—a fact indicating a higher content of chronic toxicants.

Table 8 presents data from tests on mustard, fiddleneck, and carrots; straight-run and cracked gasolines were used, and the first fraction of stove oil is added for comparison. Here, again, the cracked gasoline is most toxic; in fact, it damaged carrots considerably. The sample of straight-run gasoline at 15 ml per culture also injured carrots, whereas the stove-oil fraction was noninjurious. These results contrast with the data in table 2 (p. 86) on tests in which 24 ml of gasoline failed to cause injury.

Table 9 gives data for the same three fractions on grass and flax. Grass is the most seriously injured, and cracked gasoline is the most toxic fraction. Flax, which is intermediate in tolerance of oil toxicity, was severely injured by these fractions—a fact indicating that the acute toxicants which they carry are not selective on this crop.

TABLE 8
TOXICITY OF STRAIGHT-RUN AND CRACKED GASOLINES AND STOVE OIL TO WEEDS*
AND CARROTS; NOVEMBER 3, 1944

Oil fraction and amount applied per culture	Injury to weeds (W) and carrots (C), in per cent											
	1 day		2 days		3 days		4 days		5 days		7 days	
	W	C	W	C	W	C	W	C	W	C	W	C
Straight-run gasoline:												
3 ml	60	0	75	0	85	0	90	0	90	0	90	0
4 ml	75	0	85	0	95	0	95	0	95	0	100	0
5 ml	90	0	95	0	98	0	99	0	99	0	100	0
6 ml	90	0	95	0	99	0	100	0	100	0	100	0
8 ml	95	0	99	0	100	0	100	0	100	0	100	0
10 ml	95	0	99	0	100	0	100	0	100	0	100	0
12 ml	98	0	99	0	100	0	100	0	100	0	100	0
15 ml	99	0	99	0	100	10	100	10	100	10	100	10
Cracked gasoline:												
3 ml	75	0	90	0	98	0	100	0	100	0	100	0
4 ml	85	0	95	0	99	0	100	0	100	0	100	0
5 ml	95	0	98	0	100	0	100	0	100	0	100	0
6 ml	95	0	99	0	100	0	100	0	100	0	100	0
8 ml	97	0	99	0	100	0	100	0	100	0	100	0
10 ml	99	0	100	10	100	10	100	10	100	10	100	5
12 ml	99	0	100	25	100	25	100	25	100	25	100	15
15 ml	99	0	100	40	100	40	100	40	100	40	100	30
Stove oil no. 1:												
3 ml	70	0	85	0	85	0	85	0	85	0	80	0
4 ml	85	0	90	0	95	0	98	0	99	0	100	0
5 ml	90	0	95	0	98	0	99	0	100	0	100	0
6 ml	95	0	98	0	100	0	100	0	100	0	100	0
8 ml	98	0	99	0	100	0	100	0	100	0	100	0
10 ml	99	0	100	0	100	0	100	0	100	0	100	0
12 ml	99	0	100	0	100	0	100	0	100	0	100	0
15 ml	100	0	100	0	100	0	100	0	100	0	100	0

* Weeds referred to here were *Brassica* and *Amsinckia* species.

Kerosene and Mineral Seal Oil. Testing of additional commercial oil fractions was carried out with a twofold objective: to find a nontoxic diluent for use in applying compounds of known toxicity; and to discover, if possible, further selectivities for use in field practice. At the outset of the oil studies, a large sample of odorless kerosene was provided as a nontoxic diluent. Probably it had been solvent-extracted (Edeleanu process) and heavily acid-treated; supposedly it was inert toward plant growth. Another, heavier fraction, called mineral seal oil, which had received similar treatment, was also suggested as a diluent. Table 10 (p. 98) presents data on tests of odorless kerosene, common kerosene, and mineral seal oil. These tests accompanied those reported, in table 4, on gasoline, stove oil, and Diesel oil.

As table 10 shows, kerosene had a slight acute toxicity on the mixed weed cultures, but the plants grew out of this injury by the end of the 24-day period of observation. Against grass, on the other hand, there developed after about 5 days a toxicity that increased rapidly with time and that finally killed the cultures. Carrots and flax were immune to this damage. Figure 2, *B*, illustrates the effects of chronic injury by kerosene.

TABLE 9

TOXICITY OF STRAIGHT-RUN GASOLINE, CRACKED GASOLINE, AND STOVE-OIL FRACTION NO. 1 TO GRASS AND FLAX; NOVEMBER 3, 1944

Oil fraction and amount applied per culture	Injury to grass (G) and flax (F), in per cent											
	1 day		2 days		3 days		4 days		5 days		7 days	
	G	F	G	F	G	F	G	F	G	F	G	F
Straight-run gasoline:												
2 ml.	60	40	95	60	100	60	100	60	100	60	100	60
3 ml.	60	40	95	90	95	90	99	90	100	90	100	90
4 ml.	75	80	100	95	100	100	100	100	100	99	100	99
5 ml.	90	80	100	95	100	95	100	99	100	100	100	100
6 ml.	95	90	100	98	100	100	100	100	100	100	100	100
8 ml.	95	95	100	98	100	100	100	100	100	100	100	100
Cracked gasoline:												
2 ml.	75	50	90	75	100	75	100	75	100	75	100	85
3 ml.	90	80	100	95	100	98	100	98	100	99	100	99
4 ml.	95	90	100	98	100	100	100	100	100	100	100	100
5 ml.	100	100	100	100	100	100	100	100	100	100	100	100
6 ml.	100	100	100	100	100	100	100	100	100	100	100	100
8 ml.	100	100	100	100	100	100	100	100	100	100	100	100
Stove oil no. 1:												
2 ml.	75	60	100	90	100	90	100	90	100	90	100	90
3 ml.	75	75	100	90	100	90	100	90	100	90	100	90
4 ml.	85	80	100	95	100	95	100	95	100	98	100	98
5 ml.	95	90	100	95	100	95	100	98	100	98	100	99
6 ml.	98	95	100	95	100	98	100	99	100	100	100	100
8 ml.	98	95	100	98	100	100	100	100	100	100	100	100

Odorless kerosene lacked acute toxicity, but produced chronic toxicity somewhat more rapidly on grass than did the plain kerosene; some injury was observed on the fifth day. Evidently the refining treatment did not prevent the oil from causing this type of injury.

Although mineral seal oil had no initial effect, it caused, after 5 days, a chronic injury, which increased with time and damaged all the species tested. Figure 3, *A*, shows the results of spraying carrots, barley, and flax with mineral seal oil.

Kerosene having a boiling range of 350° to about 470° F is somewhat lighter than stove oil. Furthermore, the aromatic and olefinic content is less in kerosene owing to the refining procedures ordinarily used. This latter fact may explain its lack of toxicity to carrots. In table 10, kerosene appears too toxic to serve as a diluent where grasses and mixed weeds are the test plants. Odorless kerosene is also of questionable value for this purpose.



Fig. 3.—*A*, Carrot, barley, and flax cultures sprayed with mineral seal oil. The barley succumbed to chronic toxicity; the flax was partially injured; carrots were uninjured when photographed but later many of the plants died. *B*, Similar cultures sprayed with normal cetane. At this stage a clustering of the leaves of the flax due to foreshortening of the internodes is the only symptom. Later, the flax recovered and grew normally; the barley became heavily infected with mildew and growth was retarded.

According to the results reported in tables 4, 5, and 10, carrots tolerate the aromatic and olefinic compounds with boiling points up to 424° F or even higher that occur naturally in petroleum fractions from western crudes. Higher-boiling fractions containing aromatics and olefins are

toxic; but if these unsaturates are reduced in concentration by refining processes, the remaining oil becomes again nontoxic to carrots though still toxic to grass. This is the case with kerosene.

Grasses are apparently much more susceptible to oil injury, and both samples of kerosene were toxic to these plants. Toxicity of the heavy mineral seal oil to all plants was chronic in type. It may result from the small content of aromatic and olefinic material remaining in the oil after the solvent extraction and the heavy acid treatment. Since all plant species in the test were injured, perhaps the toxicants of oils in this range are lacking in selectivity. The wide differential between grass and flax with respect to toxicity from kerosene, on the other hand, promises a method for destroying wild oats in flax, a practical problem.

The refinement of fuel oils, especially Diesel oil, decreases the toxicity; and any refining processes that remove aromatic compounds would undoubtedly reduce the herbicidal effect of such oil.

*B*

Heavy Fuel Oil. One attempt to counteract this tendency has been the mixing of heavier, less refined fuel oil with Diesel oil. Though this causes little or no increase in acute toxicity, it apparently enhances chronic toxicity and makes the results of oil spraying more lasting. Field observations have shown, furthermore, that where such a mixture of oils has been applied heavily on hoary cress, oil seepage into and around the crowns has resulted in killing the roots to as much as 12 inches below ground level.

To fortify Diesel oil, some have used U.S. no. 1 heavy fuel oil having a gravity of 14.1°.

Table 11 shows the viscosities of several blends of U.S. no. 1 fuel oil with a Diesel oil whose A.P.I. gravity rating is 32.3. These mixtures are all more difficult to spray than straight Diesel. The one-sixth and one-fourth U.S. fuel-oil mixtures are fluid enough to be handled readily in the field, but the heavier mixes do not cover well.

U.S. no. 1 fuel oil and Diesel oil were used in varying concentrations to spray grass cultures in the greenhouse. Table 12 presents the results. The grasses, being rather mature, were difficult to kill. The relative toxicities, however, indicate the properties of the oils.

This heavy fuel oil did not atomize well; the droplets that came from the sprayer gave the plants a speckled appearance. With time, however, these droplets spread so that after a day the plants had a uniform dark coating. The dilutions all covered well at the time they were applied.

As table 12 shows, the combination of one part of fuel oil with 3 parts of odorless kerosene was the most toxic of the lot. Evidently the heavy oil did

not penetrate readily, whereas this dilution covered faster, penetrated well, and produced the greatest injury. This combination resembled Diesel oil in physical properties and approached it most nearly in toxicity. The injury

TABLE 10
TOXICITY OF ODORLESS KEROSENE, KEROSENE, AND MINERAL SEAL OIL TO WEEDS,*
GRASS, CARROTS, AND FLAX; FEBRUARY 28, 1944

Oil fraction and amount applied per culture	Injury to weeds (W), grass (G), carrots (C), and flax (F), in per cent															
	1 day				2 days				3 days				5 days			
	W	G	C	F	W	G	C	F	W	G	C	F	W	G	C	F
Kerosene:																
7.5 ml.	2	0	0	0	2	0	0	0	4	0	0	0	4	0	0	0
15.0 ml.	1	0	0	0	2	0	0	0	2	0	0	0	4	5	0	0
Odorless kerosene:																
7.5 ml.	0	0	0	0	0	0	0	0	0	0	0	0	2	10	0	0
15.0 ml.	0	0	0	0	0	0	0	0	0	0	0	0	5	20	0	0
Mineral seal oil:																
7.5 ml.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	10	0
15.0 ml.	0	0	0	0	0	0	0	0	0	0	0	0	2	0	5	0
	7 days				11 days				15 days				24 days			
	W	G	C	F	W	G	C	F	W	G	C	F	W	G	C	F
Kerosene:																
7.5 ml.	0	10	0	0	0	50	0	0	0	75	0	0	0	100	0	0
15.0 ml.	4	50	0	0	4	75	0	0	4	95	0	0	0	100	0	0
Odorless kerosene:																
7.5 ml.	2	50	0	0	2	90	0	0	2	100	0	0	0	100	0	0
15.0 ml.	6	90	0	0	4	100	0	0	10	100	0	0	0	100	0	0
Mineral seal oil:																
7.5 ml.	2	0	15	5	2	10	10	10	7	20	10	25	30	30	50	25
15.0 ml.	4	5	10	5	9	20	10	50	20	40	30	100	30	60	75	100

* Weeds referred to here were *Brassica*, *Amsinckia*, and *Lactuca* species.

TABLE 11
VISCOSITY, FLASH POINT, AND GRAVITY OF BLENDS OF DIESEL AND FUEL OILS

Blend	Viscosity, Saybolt, in seconds				Flash point, P.M. c.c., ° F	Gravity ° A.P.I.
	At 70° F	At 100° F	At 125° F	At 150° F		
Diesel oil.	44.2	38.0	35.4	33.3	180	32.3
Diesel oil, 5 parts; U.S. fuel oil no. 1, 1 part.	56.0	43.8	38.5	35.8	182	29.0
Diesel oil, 3 parts; U.S. fuel oil no. 1, 1 part.	65.5	48.9	42.1	37.9	184	27.2
Diesel oil, 1 part; U.S. fuel oil no. 1, 1 part.	118.0	71.1	56.2	47.2	188	22.3

was chronic and, with the heavy oil, developed only after several days. Had the test plants been young succulent barley, the general level of toxicity would have been higher; but the relative responses would not have been different.

Isoparaffins. Continued search for a nontoxic diluent led to the use of two isoparaffinic fractions that were reputedly 100 per cent unsulfonated. These

were synthetic oils, polymerized from light aliphatic hydrocarbons and presumably free of aromatic and olefinic compounds. Table 13 shows the toxicity produced by these fractions, with that of other previously tested fractions

TABLE 12
TOXICITY OF U.S. No. 1 FUEL OIL AND DIESEL OIL TO MIXED GRASSES;
MARCH 9, 1944

Kind of oil and amounts of oil and diluent (odorless kerosene) applied per culture	Injury to grasses, in per cent									
	1 day	2 days	4 days	5 days	9 days	11 days	14 days	19 days	22 days	26 days
Fuel oil:										
16 ml FO, 0 ml OK....	0	0	0	0	10	25	40	40	50	50
8 ml FO, 8 ml OK....	0	0	0	5	25	50	60	60	65	65
4 ml FO, 12 ml OK...	0	0	5	15	60	85	90	95	98	98
2 ml FO, 14 ml OK...	0	0	10	25	60	90	90	75	80	80
1 ml FO, 15 ml OK...	0	0	20	50	70	90	90	70	70	70
Diesel oil:										
16 ml DO, 0 ml OK....	75	85	90	95	95	98	98	98	95	95
8 ml DO, 8 ml OK....	25	50	75	85	90	95	95	95	90	85
4 ml DO, 12 ml OK....	10	20	50	65	75	85	85	85	75	75
2 ml DO, 14 ml OK..	0	5	10	20	25	30	40	40	40	40
1 ml DO, 15 ml OK..	0	0	0	5	5	10	10	20	20	20

TABLE 13
TOXICITY OF ISOPARAFFINIC AND OTHER OIL FRACTIONS TO BARLEY;
APRIL 18, 1944

Age of plants and oil fraction	Injury to barley, in per cent				
	1 day	2 days	4 days	7 days	9 days
Young barley:					
Light isoparaffin.	0	25	40	100	100
Heavy isoparaffin.	0	0	5	20	40
Odorless kerosene.	0	10	25	100	100
White gasoline.	60	100	100	100	100
Stove oil.	75	100	100	100	100
Diesel oil.	30	75	95	100	100
Old barley—8 inches tall:					
Light isoparaffin.	0	0	0	30	60
Heavy isoparaffin.	0	0	0	0	20
Odorless kerosene.	0	0	0	25	90
White gasoline.	10	20	50	75	85
Stove oil.	10	40	60	95	100
Diesel oil.	0	0	50	95	100

included for comparison. Tests were made on barley because that plant has proved most sensitive to oil.

Although these fractions were low in toxicity, they definitely injured barley. The light fraction was about twice as toxic as the heavy, and the injury was more like the acute effects of gasoline. White gasoline and stove oil caused severe acute injury; Diesel oil, a chronic injury that proved fatal by the end of the test period.

ACID TREATMENT AND SOLVENT EXTRACTION

As has long been recognized, the toxicity of oils to plants can be reduced by acid treatment and other refining processes (Gray and de Ong, 1926). These treatments remove aromatic and olefinic fractions as well as sulfur and nitrogen compounds. Presumably, the unsulfonated residues of heavy acid treatment and the raffinates from Edeleanu extraction are composed largely of aliphatic and naphthenic hydrocarbons.

Unsulfonated Residues of Stove Oil and Diesel Oil. Unsulfonated residues of stove oil and Diesel oil were prepared and tested in comparison with the untreated oils. Table 14 presents the results.

TABLE 14

TOXICITY OF STOVE OIL, DIESEL OIL, AND THEIR UNSULFONATED RESIDUES ON *Brassica*, *Amsinckia*, AND OTHER WEEDS; * NOVEMBER 1, 1943

Oil fraction	Injury to <i>Brassica</i> (B), <i>Amsinckia</i> (A), and other weeds (W), in per cent								
	1 day			2 days			3 days		
	B	A	W	B	A	W	B	A	W
Stove oil	100	100	95	100	100	100	100	100	100
Stove oil, UR.....	0	10	5	0	60	5	10	90	5
Diesel oil.	60	100	40	70	100	60	90	100	85
Diesel oil, UR.....	10	10	15	10	10	15	10	20	20

* Weeds referred to here were *Stellaria* and *Lactuca* species.

Although these readings did not carry through until chronic toxicity had developed fully, they do indicate a marked reduction in toxicity as a result of the heavy acid treatment. Toxicity is not eliminated, however; and the unsulfonated residues would not serve as nontoxic diluents.

The amount of injury caused by the unsulfonated residues of the experiment just described seemed high. For this reason, similar residues from four fractions each of stove oil and Diesel oil were made ready and compared with the untreated oils. Table 15 gives the results. The toxic materials left were most concentrated in the residues of the light fractions of stove oil. The Diesel-oil residues were free from compounds that might cause injury within 7 days. The weeds involved were mustard, fiddleneck, and annual sow thistle. A test on the toxicity of the unsulfonated residues of four gasoline fractions indicated higher values for the acute toxicity of the lighter fractions, as with stove oil. Chronic toxicity was higher in the heavier fractions (table 19, p. 104).

In a more detailed study of the toxicity of stove-oil residues, some narrower cuts were prepared, and each was sulfonated. Fractions 1a and 1b in table 16 were obtained by distillation of the previous fraction 1 to separate it into two equal portions. Original fractions 2 and 3 were similarly divided. Fraction 4 was sulfonated without separation, and its residue appears as number 4 in table 16.

Table 16 confirms the observation that the lighter fractions of stove oil contain toxic materials that are not removed by sulfonation. The toxicity of the Diesel-oil residue reported in table 14 must have resulted from incomplete sul-

TABLE 15
TOXICITY OF STOVE OIL AND DIESEL OIL AND THEIR UNSULFONATED RESIDUES TO WEEDS*
AND CARROTS; NOVEMBER 17, 1943

Kind of oil and fraction number	Injury to weeds (W) and carrots (C), in per cent					
	1 day		3 days		7 days	
	W	C	W	C	W	C
Stove oil:						
No. 1	95	0	100	0	100	0
No. 2	99	0	100	0	100	0
No. 3	99	0	100	0	100	0
No. 4	99	0	100	0	100	0
Stove oil, unsulfonated residue:						
No. 1	90	0	100	0	100	0
No. 2	10	0	30	0	50	0
No. 3	0	0	0	0	5	0
No. 4	0	0	0	0	0	0
Diesel oil:						
No. 1	99	0	100	0	100	0
No. 2	99	0	100	0	100	0
No. 3	95	0	100	5	100	5
No. 4	95	0	100	20	100	20
Diesel oil, unsulfonated residue:						
No. 1	0	0	0	0	0	0
No. 2	0	0	0	0	0	0
No. 3	0	0	0	0	0	0
No. 4	0	0	0	0	0	0

* Weeds referred to here were *Brassica*, *Amsinckia*, and *Sonchus*.

TABLE 16
TOXICITY OF UNSULFONATED RESIDUES OF STOVE OIL TO *Amsinckia*, *Lactuca*,
AND GRASSES; NOVEMBER 27, 1943

Unsulfonated residues of stove oil, fraction numbers	Injury to <i>Amsinckia</i> (A), <i>Lactuca</i> (L), and grasses (G), in per cent					
	1 day			3 days		
	A	L	G	A	L	G
No. 1a	80	20	25	85	25	30
No. 1b	90	5	30	100	10	50
No. 2a	50	5	10	80	10	10
No. 2b	10	0	0	15	0	5
No. 3a	5	0	0	5	0	0
No. 3b	0	0	0	0	0	0
No. 4	0	0	0	0	0	0

fonation or from the high greenhouse temperature during the test. Although the unsulfonated residue from Diesel oil should, if properly prepared, have little or no acute toxicity, the development of chronic toxicity by mineral seal oil (table 10, p. 98) indicates that the Diesel-oil residue would act similarly if tested over a long period.

Table 17 reports a more complete test of stove oil and its unsulfonated residue. The third and fourth fractions of Diesel oil are included, since they indicate the toxicity of fractions heavier than the heavier stove-oil cuts. These

TABLE 17

TOXICITY OF STOVE-OIL FRACTIONS, DIESEL-OIL FRACTIONS, AND FRACTIONS OF THE UNSULFONATED RESIDUE OF STOVE OIL ON *Amsinckia*, *Poa*, BARLEY, *Lactuca*, AND CARROTS; DECEMBER 10, 1943

Kind of oil and fraction number	Injury to <i>Amsinckia</i> (A), <i>Poa</i> (P), barley (B), <i>Lactuca</i> (L), and carrots (C), in per cent									
	1 day					3 days				
	A	P	B	L	C	A	P	B	L	C
Stove oil, unsulfonated residue:										
No. 1a.....	90	60	70	0	0	100	95	90	0	0
No. 1b.....	90	30	30	0	0	95	60	80	0	0
No. 2a.....	50	15	10	0	0	100	70	50	5	0
No. 2b.....	10	0	0	0	0	40	5	25	10	0
No. 3a.....	0	0	0	0	0	5	0	0	10	0
No. 3b.....	0	0	0	0	0	0	0	0	10	0
No. 4.....	0	0	0	0	0	0	0	0	10	0
Stove oil:										
No. 1.....	90	90	75	50	0	100	100	90	70	5
No. 2.....	90	90	75	40	0	100	100	75	60	5
No. 3.....	90	75	60	30	0	100	100	95	50	5
No. 4.....	75	50	20	10	0	100	100	90	40	5
Diesel oil:										
No. 3.....	90	50	20	10	0	100	95	50	30	10
No. 4.....	75	50	10	0	0	100	95	30	40	20
	6 days					8 days				
	A	P	B	L	C	A	P	B	L	C
Stove oil, unsulfonated residue:										
No. 1a.....	100	100	100	5	0	100	100	100	0	0
No. 1b.....	100	70	90	5	0	100	75	100	5	0
No. 2a.....	100	90	90	5	0	100	95	100	5	0
No. 2b.....	75	5	75	10	0	90	10	100	5	0
No. 3a.....	20	0	25	10	0	30	0	40	10	0
No. 3b.....	5	0	10	10	0	20	0	20	10	0
No. 4.....	0	0	0	10	0	0	0	0	5	0
Stove oil:										
No. 1.....	100	100	100	100	5	100	100	100	100	5
No. 2.....	100	100	50	75	5	100	100	50	75	5
No. 3.....	100	100	100	60	5	100	100	100	90	5
No. 4.....	100	100	95	90	5	100	100	100	100	5
Diesel oil:										
No. 3.....	100	100	60	50	10	100	100	75	80	10
No. 4.....	100	100	60	40	50	100	100	75	80	80

data show definitely that the lighter residues of stove oil are toxic, but that different plant species respond differently to this toxicity; and they prove again the fact that Diesel-oil fractions from the heavy end cause chronic toxicity to carrots.

One short experiment was conducted to test the effect of sulfonation on the heavy isoparaffin reported in table 13. The results proved that some sul-

fonatable material accounts for the toxicity of the fraction. The unsulfonated residue of the isoparaffin displayed no toxicity within the 8-day observation period (see, however, table 18).

The storage tests reported in the section on toxicity of gasoline and of similar light fractions (p. 116 to 120) show that certain refined fractions tended to increase in toxicity. After these tests, unsulfonated residues of stored lots of stove oil and of the heavy isoparaffinic fraction were prepared. To rid the residues of a turbidity that developed during sulfonation, they

TABLE 18

TOXICITY TO FLAX, BARLEY, AND CARROTS, OF UNSULFONATED RESIDUES OF STOVE OIL AND ISOPARAFFIN EXPOSED TO LIGHT; NOVEMBER 29, 1945

Fraction designation	Injury to flax (F), barley (B), and carrots (C), in per cent											
	1 day			3 days			7 days			11 days		
	F	B	C	F	B	C	F	B	C	F	B	C
S O UR reg*	0	0†	0	0	15	0	0	25	0	0	35	0
S O UR sp†	0	0†	0	0	20	0	0	35	0	0	50	0
I P UR reg*	0	0†	0	0	0	0	0	5	0	0	10	0
I P UR sp†	0	0†	0	0	0	0	0	5	0	0	10	0
	14 days			18 days			23 days			40 days		
	F	B	C	F	B	C	F	B	C	F	B	C
S O UR reg.	0	50	0	0	60	0	0	90	0	0	100	0
S O UR sp	0	65	0	0	75	0	0	95	0	0	100	0
I P UR reg.	0	25	0	0	50	0	0	90	0	0	100	0
I P UR sp	0	25	0	0	50	0	0	90	0	0	100	0

* S O URreg was an unsulfonated residue of stove oil prepared in the usual way. I P URreg was a similar residue from the heavy isoparaffinic fraction first reported in table 13.

† S O URsp and I P URsp refer to the light clear fraction separated from portions of the regular fractions by centrifugation.

‡ Barley plants all exhibited reversed geotropism on the first day after treatment with these four fractions.

were centrifuged. The small, clear fraction which then separated out on top of the main body of the liquid was decanted and marked *URsp*. This label indicated a special fraction, whereas *URreg* designated ordinary residues like those used in previous tests. The two types of residue from both stove oil and isoparaffin were tested on flax, barley, and carrots, with the results shown in table 18. Under the conditions of the test these fractions were toxic only to barley; flax and carrots were uninjured.

The first evidence of response by barley was a reversed geotropism shown on the day after spraying. On the second day the stove-oil residues injured the barley visibly; injury gradually developed from all treatments, the barley being dead 40 days after spraying. The isoparaffin fractions were slower acting than the stove-oil residues; there was no difference between the centrifuged and regular residues. Barley sprayed with all four unsulfonated residues became severely infected with powdery mildew during the tests, and this fungus hastened the death of the plants. Unsprayed controls were only lightly infected.

Studies on the unsulfonated residues of gasoline fractions proved these to be even more toxic than those of stove oil reported in tables 1, 3, and 4 (p. 86 and 87). Results are given in table 19.

TABLE 19
TOXICITY OF UNSULFONATED RESIDUES OF GASOLINE FRACTIONS* TO GRASS AND
BROAD-LEAVED WEEDS; NOVEMBER 11, 1944

Gasoline fraction and distillation temperature	Injury to grass (G) and broad-leaved weeds (BW), in per cent									
	1 day		2 days		3 days		4 days		6 days	
	BW	G	BW	G	BW	G	BW	G	BW	G
Straight-run gasoline, 330°-430° F.	75	85	85	95	100	100	100	100	100	100
Straight-run gasoline, unsulfonated residue:										
No. 1, 300°-345° F.	50	75	50	75	50	75	50	60	50	60
No. 2, 345°-383° F.	20	60	25	70	30	75	30	85	40	95
No. 3, 383°-390° F.	15	60	20	75	25	85	25	90	40	90
No. 4, 390° F.	40	75	30	85	25	95	25	98	50	95
Cracked gasoline, total unsulfonated residue	60	20	60	20	60	20	60	40	75	50

	8 days		10 days		12 days		22 days			
	BW	G	BW	G	BW	G	BW	G		
Straight-run gasoline, 330°-430° F.	100	100	100	100	100	100	100	100		
Straight-run gasoline, unsulfonated residue:										
No. 1, 300°-345° F.	50	50	50	40	40	30	40	0		
No. 2, 345°-383° F.	50	100	50	100	40	100	40	100		
No. 3, 383°-390° F.	50	95	50	95	40	95	50	100		
No. 4, 390° F.	65	100	65	100	65	100	75	100		
Cracked gasoline, total unsulfonated residue.	75	75	75	90	70	95	85	100		

* This was a special sample of straight-run gasoline supplied through the courtesy of the Shell Oil Company.

TABLE 20
TOXICITY OF EDELEANU EXTRACT, TECHNICAL TOLUENE SOLVENT, GASOLINE, AND
KEROSENE TO *Poa annua*, BARLEY, AND CARROTS; JANUARY 8, 1944

Oil fraction	Toxicity to <i>Poa annua</i> (P), barley (B), and carrots (C), in per cent					
	1 day			3 days		
	P	B	C	P	B	C
E-1295	100	100	100	100	100	100
TS-28.	100	100	100	100	100	100
Gasoline.	100	75	0	100	75	0
Kerosene.	0	0	0	0	0	0

Although these values are lower than those for the total gasoline fractions of tables 8 and 9 (p. 94 and 95), they are still very high for acid-treated samples. They indicate that sulfonation does not free these light fractions of toxic compounds. Evidently such compounds within this boiling range do not decompose readily under the influence of concentrated sulfuric acid.

Toxicity of the lightest unsulfonated residue was principally acute. The heavier materials contained enough of the heavier unsaturates to be lethal to grasses by the end of the 22-day observation period. The unsulfonated residue from the cracked gasoline, though low in acute toxicity, had the highest total toxicity by the end of the experiment.

Products of Solvent Extraction. Samples of Edeleanu extract,⁸ raffinate, and various products resulting from further refinement of the extract have been tested for toxicity and selectivity. Being high in aromatic and olefinic compounds, the extract is naturally high in toxicity. Table 20 shows results of a preliminary test with gasoline, kerosene, Edeleanu extract (E-1295), and a technical solvent (TS-28) produced by fractional distillation of the extract. The extracts proved extremely high in acute toxicity.

In the refinement of Edeleanu extract for the production of commercial solvents, an end product is placed on the market by the Tidewater Associated Oil Company under the name Avon Weed Killer. This material is high in aromatic and olefinic compounds. According to specifications provided by the manufacturer, it has the following physical properties:

Gravity, A.P.I.: 19.0° to 46.5°
Flash (Pensky-Martens closed cup), minimum: 150° F
Viscosity, Saybolt Universal at 100° F maximum: 50 sec.
Initial boiling point: about 400° F
Final boiling point: about 700° F

Table 21 gives results of a test comparing Edeleanu extract with Avon Weed Killer at various concentrations, odorless kerosene being used as a diluent. Both products are high in acute toxicity; but this drops off rapidly with dilution, especially on carrots. The Avon Weed Killer, being heavier than the original extract, has a somewhat higher chronic toxicity.

More detailed information on the toxicity of these products is contained in table 22, which compares four fractions of gasoline, E-1295, and Avon Weed Killer. The gasoline was used at 100 per cent and 75 per cent concentrations, whereas E-1295 and Avon were used at 25 per cent and 12.5 per cent. This difference was used to bring the gasoline into a comparable toxicity range. As these data show, the heavier fractions of all three of these products are the more toxic. The high volatility of the light fractions may partly explain these results.

To ascertain how extraction affects the toxicity of a medium-weight petroleum fraction, studies were made on a series of fractional extracts of kerosene distillate. Gravities of the extracts were as follows:

	E-1296	E-1297	E-1298	E-1299	E-1300
Per cent of feed.....	7.6	8.7	3.1	4.7	75.9
Gravity, A.P.I.	22.2°	23.0°	27.5°	32.8°	44.0°

These figures prove that the initial extraction takes out the heavier of the aromatic and olefinic compounds, the gravity shifting with successive extractions in the direction of lighter fractions.

Table 23 presents the results of these tests, the data being taken on grass and carrot cultures. As these figures show, the first extract (E-1296) was almost identical with the total extract (E-1295); the second extract was almost iden-

⁸ See footnote 9, p. 112.

TABLE 21

TOXICITY OF EDELEANU EXTRACT AND AVON WEED KILLER TO CARROTS, GRASS,
AND *Chenopodium murale*; FEBRUARY 3, 1944

Kind of oil and amounts of oil and diluent (odorless kerosene) applied per culture	Injury to carrots (C), grass (G), and <i>Chenopodium murale</i> (Cm), in per cent														
	2 days			4 days			8 days			12 days			18 days		
	C	G	Cm	C	G	Cm	C	G	Cm	C	G	Cm	C	G	Cm
Edeleanu extract:															
10 ml E, 0 ml OK . . .	75	95	95	95	100	100	100	100	100	100	100	100	100	100	100
5 ml E, 5 ml OK . . .	25	95	95	25	100	100	20	100	100	20	100	100	25	100	100
2½ ml E, 7½ ml OK . . .	0	95	90	0	100	100	0	100	100	0	100	100	5	100	100
1¼ ml E, 8¾ ml OK . . .	0	70	90	0	75	100	0	90	100	0	95	100	0	97	100
½ ml E, 9½ ml OK . . .	0	15	20	0	40	40	0	60	60	0	70	60	0	80	50
Avon Weed Killer:															
10 ml A, 0 ml OK . . .	50	95	95	75	100	100	100	100	100	100	100	100	100	100	100
5 ml A, 5 ml OK . . .	10	95	95	25	100	100	25	100	100	25	100	100	25	100	100
2½ ml A, 7½ ml OK . . .	0	90	90	5	100	100	5	100	100	5	100	100	10	100	100
1¼ ml A, 8¾ ml OK . . .	0	30	75	0	60	95	0	100	50	0	95	100	5	98	100
½ ml A, 9½ ml OK . . .	0	10	20	0	30	90	0	75	20	0	75	95	0	80	100

TABLE 22

TOXICITY OF OIL FRACTIONS TO *Malva*, AND GRASSES;
FEBRUARY 23, 1944

Oil fraction and amounts of fraction (F) and diluent (odorless kerosene) applied per culture	Injury to <i>Malva</i> (M) and grasses (G), in per cent											
	1 day		2 days		3 days		5 days		7 days		12 days	
	M	G	M	G	M	G	M	G	M	G	M	G
Gasoline:												
No. 1, 10 ml F, 0 ml OK . . .	60	20	60	20	70	20	75	25	70	20	75	25
No. 1, 7½ ml F, 2½ ml OK . . .	40	10	40	10	50	10	50	20	40	10	40	20
No. 2, 10 ml F, 0 ml OK . . .	90	90	95	95	99	97	99	100	99	99	100	100
No. 2, 7½ ml F, 2½ ml OK . . .	80	75	90	85	95	90	95	90	90	90	90	95
No. 3, 10 ml F, 0 ml OK . . .	95	90	100	95	100	99	100	100	100	100	100	100
No. 3, 7½ ml F, 2½ ml OK . . .	80	90	80	95	80	100	90	100	90	100	90	100
No. 4, 10 ml F, 0 ml OK . . .	100	100	100	100	100	100	100	100	100	100	100	100
No. 4, 7½ ml F, 2½ ml OK . . .	80	95	80	95	85	98	85	100	85	100	90	100
Edeleanu extract:												
No. 1, 2½ ml F, 7½ ml OK . . .	65	50	70	75	75	80	75	85	70	90	75	100
No. 1, 1¼ ml F, 8¾ ml OK . . .	25	5	30	10	40	20	40	20	40	20	50	25
No. 2, 2½ ml F, 7½ ml OK . . .	80	65	80	90	90	95	90	100	90	100	90	100
No. 2, 1¼ ml F, 8¾ ml OK . . .	50	20	50	10	55	20	65	40	75	40	75	80
No. 3, 2½ ml F, 7½ ml OK . . .	90	75	90	95	95	100	97	100	97	100	97	100
No. 3, 1¼ ml F, 8¾ ml OK . . .	70	40	70	40	70	60	75	75	75	80	75	95
No. 4, 2½ ml F, 7½ ml OK . . .	100	100	100	100	100	100	100	100	100	100	100	100
No. 4, 1¼ ml F, 8¾ ml OK . . .	95	75	100	75	100	90	100	90	100	90	100	95
Avon Weed Killer:												
No. 1, 2½ ml F, 7½ ml OK . . .	75	60	75	90	80	95	85	100	85	100	75	100
No. 1, 1¼ ml F, 8¾ ml OK . . .	50	30	50	40	50	50	65	75	75	75	60	90
No. 2, 2½ ml F, 7½ ml OK . . .	90	75	90	95	97	99	100	100	100	100	100	100
No. 2, 1¼ ml F, 8¾ ml OK . . .	75	50	75	60	75	75	75	75	75	75	75	90
No. 3, 2½ ml F, 7½ ml OK . . .	95	85	99	95	100	100	100	100	100	100	100	100
No. 3, 1¼ ml F, 8¾ ml OK . . .	85	70	90	75	95	85	85	90	95	90	90	95
No. 4, 2½ ml F, 7½ ml OK . . .	100	95	100	100	100	100	100	100	100	100	100	100
No. 4, 1¼ ml F, 8¾ ml OK . . .	95	85	95	90	98	95	100	100	100	100	100	100

TABLE 23

TOXICITY OF FRACTIONAL EXTRACTS OF KEROSENE DISTILLATE ON GRASS AND CARROTS;
JANUARY 15, 1944

Refinery fraction designa- tion	Injury to carrots, in per cent				Injury to grass, in per cent																	
	Pure fraction				Pure fraction				1 part fraction, 1 part OK				1 part fraction, 3 parts OK					1 part fraction, 7 parts OK				
	1 day	2 days	*3 days	10 days	1 day	2 days	*3 days	10 days	1 day	2 days	†3 days	10 days	1 day	2 days	3 days	5 days	10 days	1 day	2 days	3 days	5 days	10 days
	100	100	100	100	100	100	100	100	100	100	100	100	95	100	100	100	100	25	50	50	60	75
E-1295...	100	100	100	100	100	100	100	100	100	100	100	100	75	100	100	100	100	25	50	50	60	75
E-1296...	100	100	100	100	100	100	100	100	100	100	100	100	90	100	100	100	100	50	75	50	70	75
E-1297...	100	100	100	100	100	100	100	100	100	100	100	100	90	100	100	100	100	50	75	50	70	75
E-1298...	50	95	100	100	90	100	100	100	90	100	100	100	80	95	95	95	98	10	50	30	30	60
E-1299...	90	95	100	100	90	100	100	100	75	100	95	100	80	90	80	90	90	10	10	10	20	40
E-1300...	0	0	0	0	10	10	0	5	5	5	0	5	0	0	0	0	0	0	0	0	0	0
TS-28 ..	100	100	100	100	100	100	100	100	90	100	100	100	90	100	100	100	98	20	40	20	20	20

* Injury at 5 days was the same as at 3 days.

† Injury at 5 days was the same as at 3 days except for fraction E-1299, which showed 100 per cent injury

TABLE 24

TOXICITY OF TAR OIL RESIDUES TO FOXTAIL SOD; JANUARY 27, 1945

Amount of residue (R) and diluent (isoparaffin) applied per culture	Injury to foxtail, in per cent													
	Crude still residue: isoparaffin mixtures							Pure still residue: isoparaffin mixtures						
	1 day	2 days	3 days	4 days	6 days	9 days	13 days	1 day	2 days	3 days	4 days	6 days	9 days	13 days
6 ml R, 0 ml IP.....	100	100	100	100	100	100	100	25	90	98	98	98	98	99
3 ml R, 3 ml IP.	100	100	100	100	100	100	100	15	80	95	95	98	98	99
1½ ml R, 4½ ml IP.....	25	50	75	85	95	98	100	5	50	90	95	95	98	99
¾ ml R, 5¼ ml IP.	0	5	5	10	15	20	20	0	20	80	90	95	98	99
¾ ml R, 5¼ ml IP.	0	0	2	5	5	10	10	0	10	50	85	85	90	90
5/16 ml R, 5 5/16 ml IP.	0	0	1	1	2	10	10	0	5	25	50	50	75	75
5/32 ml R, 5 29/32 ml IP.	0	0	1	1	2	10	10	0	0	5	10	20	60	75
5/64 ml R, 5 41/64 ml IP.	0	0	1	1	2	10	10	0	0	1	10	20	60	75

tical with the first; and the extracts that followed were successively weaker in herbicidal effect. The final raffinate (E-1300), which resembled odorless kerosene in properties, was nontoxic to carrots and only slightly injurious to grass. The TS-28 solvent, a highly aromatic fraction, was of the same order of toxicity as the total extract (E-1295) and the first extract (E-1296). Boiling points and gravities on some of these fractions were as follows:

	TS-28	E-1295	Odorless kerosene	Mineral seal oil
Gravity, ° A.P.I.....	32.8	23.3	43.9	31.9
Initial boiling point, ° F.....	312.0	376.0	382.0	492.0
Final boiling point, ° F.....	408.0	516.0	481.0	648.0

Whereas these unsaturated compounds are highly toxic, when diluted they are less toxic; and any mixture containing less than about 25 per cent is too

dilute to be useful as a herbicide. The pure fractions killed carrots; the mixtures containing 12.5 per cent of the extracts were low in toxicity to carrots and not injurious enough to grass to make effective herbicides.

Coke-Still Residues. Two additional fractions high in aromatic compounds were tested for toxicity. These were (1) crude still residue, a dark fluid obtained from the coke ovens at Fontana, California; and (2) pure still

TABLE 25

TOXICITY OF ACID-TREATED GASOLINE SAMPLES TO FOXTAIL SOD; ATTEMPTED SEPARATION OF OLEFINS AND AROMATICS; JANUARY 27, 1945

Fraction and amounts of fraction (F) and diluent (isoparaffin) applied per culture	Injury to foxtail sod, in per cent						
	1 day	2 days	3 days	4 days	6 days	9 days	13 days
E-1380:							
6 ml F, 0 ml IP.	100	100	100	100	100	100	100
3 ml F, 3 ml IP.	90	95	95	95	100	100	100
1.5 ml F, 4.5 ml IP.	10	10	10	10	15	25	40
0.8 ml F, 5.2 ml IP.	0	0	5	5	10	15	15
0.4 ml F, 5.6 ml IP.	0	0	5	5	5	10	10
0.2 ml F, 5.8 ml IP.	0	0	2	2	5	10	10
0.1 ml F, 5.9 ml IP.	0	0	1	1	5	10	10
0.05 ml F, 5.95 ml IP.	0	0	1	1	5	10	10
E-1380 minus olefins:							
6 ml F, 0 ml IP.	0	10	20	30	50	75	95
3 ml F, 3 ml IP.	0	5	10	15	30	60	60
1.5 ml F, 4.5 ml IP.	0	2	5	10	15	15	15
0.8 ml F, 5.2 ml IP.	0	0	5	10	10	15	15
0.4 ml F, 5.6 ml IP.	0	0	2	5	5	10	15
0.2 ml F, 5.8 ml IP.	0	0	1	2	5	10	15
0.1 ml F, 5.9 ml IP.	0	0	1	1	5	10	10
0.05 ml F, 5.95 ml IP.	0	0	1	1	5	10	10
E-1380 minus olefins and aromatics:							
6 ml F, 0 ml IP.	0	5	15	30	50	75	80
3 ml F, 3 ml IP.	0	5	10	15	30	60	60
1.5 ml F, 4.5 ml IP.	0	2	5	10	15	30	30
0.8 ml F, 5.2 ml IP.	0	0	5	10	10	10	10
0.4 ml F, 5.6 ml IP.	0	0	2	2	5	10	15
0.2 ml F, 5.8 ml IP.	0	0	1	1	5	10	10
0.1 ml F, 5.9 ml IP.	0	0	1	1	5	10	10
0.05 ml F, 5.95 ml IP.	0	0	1	1	5	10	10
Isoparaffin alone:							
6 ml IP, first check.	0	0	1	1	5	10	10
6 ml IP, second check.	0	0	1	1	5	10	10

residue, a partially refined product from the same source. Being coal-tar residues, these materials were highly aromatic and extremely toxic. Results of the tests (table 24) indicate a very interesting situation. By refinement this residue acquired chronic toxicity, so that it was still highly toxic at a dilution of 1 part in 128 after a 13-day observation period. Since this refined fraction lacked somewhat the acute toxicity of the crude residue, conceivably the refining had removed some of the lighter aromatics and thus had increased in concentration those heavier compounds responsible for chronic injury.

Olefinic and Aromatic Compounds. One experiment involved an attempt (1) to remove olefinic compounds from a straight-run gasoline; and (2) to

remove both olefins and aromatic compounds. The methods of refinement involved use of acids of two concentrations and is based upon the assumption that olefins break down more readily than aromatics under acid treatment.

TABLE 26
TOXICITY OF REFINED-OIL SEPARATES TO GRASSES AND WEEDS;*
NOVEMBER 11, 1944

Oil fraction	Injury to weeds (W) and grasses (G), in per cent							
	1 day		2 days		3 days		4 days	
	W	G	W	G	W	G	W	G
Heavy isoparaffin.....	0	0	0	0	0	0	0	0
Heavy naphthene.....	10	20	0	0	0	0	0	0
Heavy olefins.....	90	80	95	85	95	90	95	90
Heavy aromatics.....	95	95	98	98	100	100	100	100
Naphthone A.....	100	100	100	100	100	100	100	100
High-sulfur gasoline.....	95	95	98	98	98	100	100	100

* Weeds referred to here were *Amsinckia*, *Lactuca*, and *Stellaria* species.

TABLE 27
TOXICITY OF REFINED-OIL SEPARATES TO FOXTAIL SOD; JANUARY 29, 1945

Oil fraction and amounts of oil and diluent (isoparaffin) applied per culture	Injury to plants, in per cent				
	1 day	2 days	4 days	7 days	11 days
Heavy isoparaffin:					
6 ml F, 0 ml IP.....	0	0	0	0	0
3 ml F, 3 ml IP.....	0	0	0	0	0
1½ ml F, 4½ ml IP.....	0	0	0	0	0
Heavy naphthene:					
6 ml F, 0 ml IP.....	25	25	25	35	20
3 ml F, 3 ml IP.....	10	10	10	10	5
1½ ml F, 4½ ml IP.....	0	0	5	5	5
Heavy olefin:					
6 ml F, 0 ml IP.....	50	75	90	95	95
3 ml F, 3 ml IP.....	50	75	90	95	95
1½ ml F, 4½ ml IP.....	25	60	75	85	90
Heavy aromatic:					
6 ml F, 0 ml IP.....	95	95	100	100	100
3 ml F, 3 ml IP.....	90	95	100	100	100
1½ ml F, 4½ ml IP.....	90	95	100	100	100
Naphthone A:					
6 ml F, 0 ml IP.....	100	100	100	100	100
3 ml F, 3 ml IP.....	100	100	100	100	100
1½ ml F, 4½ ml IP.....	100	100	100	100	100
High-sulfur gasoline:					
6 ml F, 0 ml IP.....	90	95	95	95	95
3 ml F, 3 ml IP.....	0	0	0	0	0
1½ ml F, 4½ ml IP.....	0	0	0	0	0

Results, as shown in table 25, indicate that the treatment probably removes most of the lighter compounds responsible for acute toxicity. The results of the second treatment, designed to remove aromatics plus olefins, are so nearly like those of the initial olefin-removing process that one doubts the effectiveness of this separation.

A more accurate evaluation of the toxic properties of the various classes of compounds found in petroleum was attempted with fractions furnished by the Tidewater Associated Oil Company. These were all heavy fractions and were so prepared that each contained predominately one type of com-

TABLE 28

TOXICITY OF HEAVY AROMATIC AND OLEFINIC COMPOUNDS TO CARROTS, FLAX,
Brassica, AND GRASS; MARCH 19, 1945

Type of fraction and per cent of fraction (F) and diluent (isoparaffin)*	Injury to carrots (C), flax (F), <i>Brassica</i> (B), and grass (G), in per cent											
	1 day				2 days				3 days			
	C	F	B	G	C	F	B	G	C	F	B	G
Heavy olefin:												
50% F, 50% IP...	50	100	100	50	70	100	100	90	75	100	100	95
45% F, 55% IP...	45	100	100	40	50	100	100	90	50	100	100	85
40% F, 60% IP...	30	95	90	30	40	100	95	75	40	100	100	85
35% F, 65% IP...	20	90	80	30	20	100	85	60	20	100	95	75
30% F, 70% IP...	10	20	80	20	10	60	80	40	10	80	95	60
Heavy aromatic:												
50% F, 50% IP...	75	90	100	75	90	100	100	95	90	100	100	100
45% F, 55% IP...	50	100	100	60	75	100	100	95	75	100	100	100
40% F, 60% IP...	40	100	100	60	60	100	100	90	60	100	100	95
30% F, 70% IP...	10	100	90	60	30	100	100	90	30	100	98	95
20% F, 80% IP...	5	100	80	50	10	100	90	75	10	100	95	90
10% F, 90% IP...	0	10	20	10	0	25	40	30	0	40	50	50
	7 days				9 days				12 days			
	C	F	B	G	C	F	B	G	C	F	B	G
Heavy olefin:												
50% F, 50% IP...	50	99	100	98	50	98	100	99	30	95	100	100
45% F, 55% IP...	40	99	100	100	40	98	100	100	20	95	100	100
40% F, 60% IP...	30	99	100	95	30	98	100	95	15	95	100	95
35% F, 65% IP...	10	99	95	90	5	98	98	95	5	90	100	95
30% F, 70% IP...	5	95	95	85	0	75	98	90	0	75	100	95
Heavy aromatic:												
50% F, 50% IP...	90	90	100	100	90	90	100	100	80	90	100	100
45% F, 55% IP...	80	99	100	100	80	98	100	100	70	95	100	100
40% F, 60% IP...	60	99	100	100	60	98	100	100	50	95	100	100
30% F, 70% IP...	20	99	100	100	20	98	100	100	10	95	100	100
20% F, 80% IP...	0	98	100	95	0	95	100	98	0	95	100	99
10% F, 90% IP...	0	50	75	75	0	50	75	80	0	40	75	85

* Total volume applied to each culture, 6 ml. -

pound. Included were samples of isoparaffinic, naphthenic, aromatic, and olefinic compounds. Also tested was a fraction termed naphthone A and a sample of high-sulfur gasoline. Table 26 presents a preliminary test, and table 27 contains more detailed information.

Judging from these data, the isoparaffinic fraction was lacking in toxicity. This fraction was considerably heavier than the previously tested heavy isoparaffin submitted as a possible diluent. It appeared even heavier than the mineral seal oil. The naphthenic fraction, though toxic, did not carry the killing power to exterminate the grasses, which therefore eventually recovered.

TABLE 29

TOXICITY OF HEAVY OLEFINIC AND AROMATIC FRACTIONS IN *n*-CETANE TO CARROTS, FLAX, AND GRASS; APRIL 25, 1945

Type of fraction and per cent of fraction (F) and diluent (<i>n</i> -cetane)*	Injury to carrots (C), flax (F), and grass (G), in per cent																	
	1 day			2 days			3 days			4 days			6 days			8 days		
	C	F	G	C	F	G	C	F	G	C	F	G	C	F	G	C	F	G
Heavy olefin:																		
50% F, 50% C...	5	20	50	10	40	75	10	50	85	20	80	98	20	90	100	10	95	100
45% F, 55% C...	5	10	30	10	30	60	20	40	70	20	60	70	20	75	80	15	90	90
40% F, 60% C...	0	5	20	5	15	40	5	20	60	10	50	50	10	65	75	10	75	80
35% F, 65% C...	0	0	10	0	5	20	0	10	30	5	25	20	0	35	30	0	40	40
30% F, 70% C...	0	0	5	0	0	10	0	2	20	0	5	20	0	10	25	0	10	20
25% F, 75% C...	0	0	0	0	0	5	0	1	10	0	5	10	0	5	15	0	5	10
Heavy aromatic:																		
40% F, 60% C...	5	50	50	10	75	75	10	85	90	10	95	100	15	95	100	15	95	100
35% F, 65% C...	0	5	60	5	10	90	5	25	90	5	75	100	5	80	100	5	80	100
30% F, 70% C...	0	0	30	5	5	60	0	15	60	0	50	80	0	70	90	0	70	95
25% F, 75% C...	0	0	15	0	5	30	0	10	40	0	25	40	0	50	75	0	60	80
20% F, 80% C...	0	0	10	0	2	20	0	5	25	0	15	25	0	20	50	0	20	60
15% F, 85% C...	0	0	5	0	0	10	0	0	15	0	0	15	0	0	20	0	0	20
10% F, 90% C...	0	0	0	0	0	5	0	0	5	0	0	5	0	0	0	0	0	0
0% F, 100% C...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mineral seal oil, 100%...	0	0	0	0	0	0	5	0	0	5	0	0	0	10	0	0	15	5
Odorless kerosene, 100%...	0	0	0	0	0	0	0	0	5	0	0	25	0	0	50	0	0	50
Kerosene, 100%...	0	0	0	0	0	0	0	0	5	0	10	0	0	0	0	0	0	0
Isoparaffin, 100%...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heavy olefin:																		
50% F, 50% C...	10	95	100	15	95	100	15	95	100	10	90	100	10	75	100			
45% F, 55% C...	15	90	95	15	90	95	10	90	100	5	80	100	5	75	100			
40% F, 60% C...	10	80	85	15	80	90	10	80	95	5	70	100	5	70	100			
35% F, 65% C...	0	50	75	5	60	80	0	60	90	0	50	100	0	50	100			
30% F, 70% C...	0	15	20	0	20	20	0	15	40	0	10	75	0	10	75			
25% F, 75% C...	0	5	10	0	5	10	0	0	20	0	0	40	0	0	50			
Heavy aromatic:																		
40% F, 60% C...	15	95	100	10	95	100	10	95	100	5	90	100	0	85	100			
35% F, 65% C...	5	80	100	5	80	100	5	90	100	0	90	100	0	90	100			
30% F, 70% C...	0	70	95	5	70	100	5	75	100	0	75	100	0	75	100			
25% F, 75% C...	0	60	85	0	60	90	0	60	90	0	60	95	0	60	100			
20% F, 80% C...	0	20	75	0	25	80	0	25	80	0	25	50	0	25	60			
15% F, 85% C...	0	0	40	0	5	40	0	0	40	0	0	20	0	0	30			
10% F, 90% C...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
0% F, 100% C...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Mineral seal oil, 100%...	0	15	10	0	15	20	0	10	50	0	10	75	0	10	90			
Odorless kerosene, 100%...	0	0	75	0	5	90	0	5	100	0	0	100	0	0	100			
Kerosene, 100%...	0	0	0	0	0	10	0	0	10	0	0	20	0	0	20			
Isoparaffin, 100%...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			

* Total volume applied to each culture, 6 ml.

The olefinic fraction was highly toxic but, like the naphthenic, lacked the power to exterminate the grasses. The aromatic fraction was very effective, and the grass cultures succumbed to its application.

The naphthone A was an odd-smelling oil of extreme toxicity. It wilted and discolored the plants rapidly after application; they all died within one day.

The high-sulfur gasoline also was toxic at full strength, but proved low in toxicity when diluted.

Because the heavy olefinic and heavy aromatic fractions seem to offer the most promise as selective toxicants, concentration series using the isoparaffin fraction as a diluent were sprayed on carrots, flax, mustard, and foxtail. Results are shown in table 28.

The aromatic fraction is somewhat more toxic than the olefinic. Both are highly selective as between carrots and other weeds. The margin of selectivity for flax is much narrower, but still may be sufficient to be of practical use.

Because the heavy isoparaffinic material used as a diluent in these experiments had a slightly chronic toxicity that began to show up after about 2 weeks, the experiment was repeated with normal cetane, a relatively nontoxic diluent. Table 29 gives these results with readings over a 22-day period. Mineral seal oil, an old sample of odorless kerosene, a fresh lot of ordinary kerosene, and a fresh sample of isoparaffin were tested as standards for comparative evaluation of toxicity. The isoparaffin and normal cetane both proved nontoxic in the tests.

These results show conclusively that the aromatic compounds are more toxic than the olefinic, if the samples used were of comparable purity. Furthermore, the aromatic fraction appears to have been somewhat more selective than the olefinic. Carrots completely recovered from the toxic effects of the higher concentrations of aromatic compounds. They showed some recovery from olefin toxicity, but not complete.

One interesting observation is the extreme effectiveness on grass of the compounds responsible for toxicity in the stale, odorless kerosene fraction. Apparently these can have constituted only a small fraction of the total makeup of the oil; yet they killed the foxtail, a result which in other tests required 25 per cent of aromatics and 35 per cent of olefinic compounds.

RELATION OF TOXICITY TO BOILING RANGE IN EDELEANU EXTRACT

Toxicity of Badger-Type Distillate Cuts. According to data cited in the preceding section, successive extracts by solvent extraction decreased in toxicity, and the final raffinate was relatively nontoxic. Another point of interest is the comparative toxicity of different temperature cuts of the Edeleanu extract.* Ten Badger-type distillation cuts were tested on grass and carrot cultures. Table 30 presents the results of tests using the cuts pure on carrots and in dilutions of 1:3 and 1:7 on grass. Odorless kerosene, which was fresh at the time of these tests, was used as a diluent.

* The Edeleanu process is the refining of oil by means of liquid sulfur dioxide to remove unsaturates.

TABLE 30

TOXICITY OF BADGER-TYPE DISTILLATION CUTS OF EDELEANU EXTRACT ON GRASS
AND CARROTS; JANUARY 15, 1944

BTD cut no.	Injury to carrots, in per cent					Injury to grass, in per cent									
	Pure cuts					With 2 parts BTD : 6 parts OK*					With 1 part BTD : 7 parts OK				
	1 day	2 days	3 days	5 days	10 days	1 day	2 days	3 days	5 days	10 days	1 day	2 days	3 days	5 days	10 days
No. 1.....	50	50	50	50	50	90	90	95	95	100	10	30	40	40	95
No. 2.....	50	50	60	50	50	75	80	90	90	95	60	75	60	75	100
No. 3.....	75	75	80	85	90	95	100	100	100	100	75	85	80	95	100
No. 4.....	90	90	100	100	100	100	100	100	100	100	85	95	90	95	100
No. 5.....	80	90	90	95	95	90	100	100	100	100	60	75	80	95	100
No. 6.....	50	75	80	85	75	95	100	100	100	100	60	90	90	95	95
No. 7.....	40	50	60	75	90	100	100	100	100	100	75	90	95	100	100
No. 8.....	90	95	100	100	100	90	100	100	100	100	60	90	98	100	100
No. 9.....	95	95	100	100	100	100	100	100	100	100	75	90	95	100	100
No. 10.....	60	90	100	100	100	90	100	100	100	100	80	98	100	100	100

* Odorless kerosene.

TABLE 31

TOXICITY OF FRACTIONAL DISTILLATES OF E-1296 AS SHOWN BY CARROTS, *Chenopodium murale*, *Malva parviflora*, AND GRASS; FEBRUARY 11, 1944

Fraction no. and amounts of fraction (F) and diluent (odorless kerosene) applied per culture	Injury to carrots (C), <i>Chenopodium murale</i> (Cm), <i>Malva parviflora</i> (M), and grass (G), in per cent											
	1 day				4 days				9 days			
	C	Cm	M	G	C	Cm	M	G	C	Cm	M	G
No. 1:												
2 ml F, 6 ml OK.....	0	50	75	10	0	95	85	10	0	100	75	5
1 ml F, 7 ml OK.....	0	5	5	0	0	15	10	0	0	20	5	0
½ ml F, 7½ ml OK.....	0	0	0	0	0	0	0	0	0	0	0	0
¼ ml F, 7¾ ml OK.....	0	0	0	0	0	0	0	0	0	0	0	0
No. 2:												
2 ml F, 6 ml OK.....	0	75	90	25	0	95	95	35	0	100	95	50
1 ml F, 7 ml OK.....	0	50	50	0	0	75	75	5	0	95	80	15
½ ml F, 7½ ml OK.....	0	0	0	0	0	5	0	0	0	10	0	0
¼ ml F, 7¾ ml OK.....	0	0	0	0	0	0	0	0	0	0	0	0
No. 3:												
2 ml F, 6 ml OK.....	0	90	90	50	0	100	95	75	0	100	95	95
1 ml F, 7 ml OK.....	0	75	75	0	0	95	85	10	0	100	65	10
½ ml F, 7½ ml OK.....	0	25	20	0	0	40	30	0	0	50	10	0
¼ ml F, 7¾ ml OK.....	0	0	0	0	0	5	0	0	0	5	0	0
No. 4:												
2 ml F, 6 ml OK.....	5	95	95	80	5	100	100	80	0	100	100	90
1 ml F, 7 ml OK.....	0	75	75	10	0	100	90	10	0	90	95	80
½ ml F, 7½ ml OK.....	0	50	20	0	0	95	30	0	0	100	40	5
¼ ml F, 7¾ ml OK.....	0	10	0	0	0	40	0	0	0	20	0	0
No. 5:												
2 ml F, 6 ml OK.....	10	95	95	90	20	100	100	100	10	100	100	100
1 ml F, 7 ml OK.....	0	90	90	50	0	100	100	50	5	100	100	75
½ ml F, 7½ ml OK.....	0	60	30	0	0	100	50	5	0	100	50	10
¼ ml F, 7¾ ml OK.....	0	20	0	0	0	75	0	0	0	100	0	0

Evidently toxicity increases with increasing weight of the cut, the heavy end being the most toxic. Boiling temperatures and gravities are shown below.

	Temperature, ° F	Gravity, ° A.P.I.
Initial boiling point.....	376	
10 per cent cut.....	394	35.3
20 per cent cut.....	403	
30 per cent cut.....	417	27.8
40 per cent cut.....	426	
50 per cent cut.....	436	25.0
60 per cent cut.....	447	
70 per cent cut.....	456	21.0
80 per cent cut.....	468	
90 per cent cut.....	484	17.1
End point.....	516	

This extract was made from kerosene distillate. The four lightest fractions were within the gravity range of Diesel oil; the remaining six were heavier. These heavy oils were highly toxic and caused complete death of carrots when used pure. At a dilution of 1:7 in kerosene they caused no injury to carrots.

Some further experiments were performed on temperature cuts of Edeleanu extracts. Table 31 presents results on five fractions of E-1296, separated by ordinary distillation, applied to cultures of carrots, goosefoot, cheeseweed, and grass. These results confirm those of table 30 and illustrate the highly selective nature of the compounds present in the extract.

TABLE 32
TOXICITY OF FOUR TEMPERATURE CUTS OF AVON WEED KILLER TO CARROTS
AND BARLEY; MAY 5, 1944

Avon fraction and amounts of fraction (F) and diluent (isoparaffin) applied per culture	Plant	Injury to carrots or barley, in per cent						
		1 day	3 days	5 days	7 days	10 days	13 days	15 days
Avon no. 1:								
10 ml F, 0 ml IP.....	Carrot	90	95	98	98	100	100	100
5 ml F, 5 ml IP.....	Carrot	30	40	40	30	30	30	40
2½ ml F, 7½ ml IP.....	Carrot	10	5	5	5	5	5	10
1¼ ml F, 8¾ ml IP.....	Carrot	0	0	0	0	0	0	0
1¼ ml F, 8¾ ml IP.....	Barley	30	30	30	30	30	40	40
Avon no. 2:								
10 ml F, 0 ml IP.....	Carrot	90	90	95	98	100	100	100
5 ml F, 5 ml IP.....	Carrot	40	50	50	60	60	60	60
2½ ml F, 7½ ml IP.....	Carrot	10	10	10	10	10	10	15
1¼ ml F, 8¾ ml IP.....	Carrot	0	0	0	0	0	0	0
1¼ ml F, 8¾ ml IP.....	Barley	40	60	60	60	60	75	75
Avon no. 3:								
10 ml F, 0 ml IP.....	Carrot	95	95	98	100	100	100	100
5 ml F, 5 ml IP.....	Carrot	50	50	90	60	70	70	70
2½ ml F, 7½ ml IP.....	Carrot	10	10	10	10	15	20	20
1¼ ml F, 8¾ ml IP.....	Carrot	0	0	0	0	0	0	0
1¼ ml F, 8¾ ml IP.....	Barley	50	75	85	90	95	100	100
Avon no. 4:								
10 ml F, 0 ml IP.....	Carrot	90	95	95	95	100	100	100
5 ml F, 5 ml IP.....	Carrot	60	60	60	60	70	75	75
2½ ml F, 7½ ml IP.....	Carrot	10	15	15	15	25	40	40
1¼ ml F, 8¾ ml IP.....	Carrot	0	0	0	0	10	15	20
1¼ ml F, 8¾ ml IP.....	Barley	90	100	100	100	100	100	100

Temperature Cuts of Avon Weed Killer. Avon Weed Killer, an even heavier material, was cut into four fractions by distillation. These fractions were applied to carrots and barley as reported in table 32. Here, again, the heavier compounds were the more toxic. Evidently the heavy olefinic and aromatic compounds of petroleum are extremely toxic to plants; furthermore, they

TABLE 33

TOXICITY OF FOUR TEMPERATURE CUTS OF KEROSENE EXTRACT (E-1296) ON BARLEY AND CHICKWEED; TWO CUTS WERE REFLUXED WITH AND WITHOUT THE PRESENCE OF AIR; JUNE 20, 1944

E-1296 cut and amounts of cut (F) and diluent (isoparaffin) applied per culture	Injury to barley and chickweed, in per cent						
	1 day	2 days	3 days	4 days	5 days	6 days	8 days
No. 1:							
6 ml F, 6 ml IP.....	50	60	75	80	85	85	85
3 ml F, 9 ml IP.....	25	30	50	60	70	70	70
1½ ml F, 10½ ml IP.....	5	10	10	10	15	15	15
No. 2:							
6 ml F, 6 ml IP.....	60	70	85	90	90	90	90
3 ml F, 9 ml IP.....	30	50	60	70	70	70	70
1½ ml F, 10½ ml IP.....	10	15	10	10	15	15	15
No. 3:							
6 ml F, 6 ml IP.....	70	80	95	95	95	95	100
3 ml F, 9 ml IP.....	35	60	70	80	85	85	90
1½ ml F, 10½ ml IP.....	15	15	15	15	15	20	20
No. 4:							
6 ml F, 6 ml IP.....	80	90	98	98	100	100	100
3 ml F, 9 ml IP.....	40	60	80	90	90	90	95
1½ ml F, 10½ ml IP.....	20	40	30	40	40	40	50
No. 2, refluxed without air:							
6 ml F, 6 ml IP.....	60	70	90	90	90	90	90
3 ml F, 9 ml IP.....	30	50	65	70	75	75	80
1½ ml F, 10½ ml IP.....	5	10	10	10	10	15	15
No. 2, refluxed with air:							
6 ml F, 6 ml IP.....	60	75	95	95	95	95	100
3 ml F, 9 ml IP.....	30	50	70	70	80	90	100
1½ ml F, 10½ ml IP.....	10	15	15	15	15	20	25
No. 3, refluxed without air:							
6 ml F, 6 ml IP.....	75	85	95	95	95	95	100
3 ml F, 9 ml IP.....	40	70	85	85	85	85	100
1½ ml F, 10½ ml IP.....	15	25	25	25	35	35	40
No. 3, refluxed with air:							
6 ml F, 6 ml IP.....	75	90	95	95	95	95	100
3 ml F, 9 ml IP.....	40	60	80	80	85	85	95
1½ ml F, 10½ ml IP.....	10	20	20	20	30	30	50

persist and bring about injury and death after the lighter compounds have vaporized and left. For complete killing of general weeds on roadsides, along railway roadbeds, and in similar situations, these heavy aromatics appear to be useful.

One additional set of experiments is reported in table 33. The results, which agree with those of tables 30, 31, and 32, indicate conclusively the relation mentioned above. The additional data of table 33 relate to the possible effects of storage upon the toxicity of oil fractions in the absence and presence of air. The gains in toxicity upon refluxing were small but perceptible.

STORAGE AND REFLUXING

Toxicity Change of Gasolines on Standing. The sample reported on in table 2 (p. 86) was a fresh lot of commercial white gasoline. The straight-run and cracked samples reported in table 8 (p. 94) were produced by the same company, but had stood in cans in the headhouse from April, when they were received, until November, when the tests were run. Various other tests had indicated that gasoline increased in toxicity upon standing. Table 34 shows tests comparing a fresh straight-run sample with a white-gasoline sample that had stood in a flint-glass bottle for over a year in the headhouse. This test proves conclusively that the 1943 gasoline sample had gained in

TABLE 34
TOXICITY OF OLD AND FRESH GASOLINE SAMPLES ON CARROTS AND WEEDS;^{*}
NOVEMBER 16, 1944

Sample and amount per culture	Injury to carrots (C) and weeds (W), in per cent							
	2 days		4 days		6 days		8 days	
	C	W	C	W	C	W	C	W
1943-sample gas, 10 ml	25	90	20	95	20	95	15	98
1944-sample gas, 10 ml.	0	98	0	100	0	100	0	100

^{*} Weeds referred to here were *Brassica*, *Amaranthus*, and *Lactuca* species.

toxicity, for it was the same sample reported in table 2 as noninjurious to carrots. Since the carrots reported in table 34 showed some recovery during the 8-day period, apparently the increase in toxicity was of the acute type. The 1944 sample was actually more toxic to the weeds.

Toxicity of Refluxed Samples. To test the possibility that oxidation is involved in the gain in toxicity of gasoline samples with time, some tests were run using gasoline fractions that had been refluxed with and without passage of air through the apparatus during the process. These fractions were compared with the unrefluxed ones. Table 6 (p. 92) reports the results. Refluxing seems to have affected toxicity slightly, if at all. Table 35 presents similar data on stove-oil fractions. In this work, again, oil refluxing had little effect on toxicity; the presence of air increased toxicity against grass slightly, but not against onions. Refluxing always caused slight darkening of the samples; and, with air, darkening was more marked.

These results show that apparently the gain in toxicity of oils upon standing involves some reaction not accelerated by temperature alone. The presence of air during refluxing caused browning of the fractions, a burned odor, and slightly more toxicity than refluxing without air.

In comparison with refluxing, standing for several weeks, particularly in the light, brings about a yellow coloration of most fractions, and the development of a stale sour odor. This is true of white gasoline and kerosene fractions; isoparaffinic fractions acquire a stale odor without change in color; stove oil undergoes no discernible change.

Toxicity of Stored Fractions. In an attempt to study further the effects of standing, tests were run on stored samples of kerosene, gasoline, stove oil, mineral seal oil, and on certain narrow fractions.

In the first test, kerosene samples were placed in clear glass bottles and stored in the light and in a dark cupboard. Six months later (November 6, 1945) they were compared with a fresh sample and with the odorless kerosene received in January, 1944, and kept in a clear glass bottle in the laboratory. Also tested were the straight-run and cracked gasoline samples received in

TABLE 35

TOXICITY OF STOVE-OIL FRACTIONS TO ONIONS AND GRASS; ONE FRACTION
REFLUXED WITH AIR AND ONE WITHOUT AIR; MAY 5, 1944

Fraction and amounts of fraction (F) and diluent (isoparaffin) applied per culture	Injury to onions (O) and grass (G), in per cent									
	1 day		3 days		5 days		10 days		15 days	
	O	G	O	G	O	G	O	G	O	G
No. 1:										
12 ml F, 0 ml IP.	70	90	60	90	50	90	25	100	15	100
6 ml F, 6 ml IP.	10	40	10	50	5	50	0	50	0	50
No. 2:										
12 ml F, 0 ml IP.	60	95	70	95	75	95	85	100	100	100
6 ml F, 6 ml IP.	10	90	10	90	0	95	0	95	0	95
No. 3:										
12 ml F, 0 ml IP.	50	90	40	95	40	95	40	100	30	100
6 ml F, 6 ml IP.	10	80	10	80	0	90	0	90	0	95
No. 4:										
12 ml F, 0 ml IP.	60	80	50	90	40	90	50	100	75	100
6 ml F, 6 ml IP.	10	70	10	70	0	75	0	75	0	75
No. 1, refluxed without air:										
12 ml F, 0 ml IP.	70	90	50	90	40	95	30	100	40	100
6 ml F, 6 ml IP.	10	40	10	50	5	50	0	50	0	50
No. 1, refluxed with air:										
12 ml F, 0 ml IP.	80	90	50	90	40	95	40	100	50	100
6 ml F, 6 ml IP.	10	60	10	60	5	75	0	75	0	75

April, 1944, the no. 3 sample of Shell white gasoline, and no. 1 sample of stove oil fractionated by the Chemistry Division in September, 1943, and a sample of the heavy isoparaffin received in April, 1944. Table 36 gives the results.

These tests show that within 6 months kerosene stored in the light will acquire a high toxicity, that the presence of air in the bottle is not essential to this increased toxicity, that gasoline samples became even more toxic than kerosene samples; that both gasoline and kerosene lost their selectivity on carrots, that the heavy isoparaffinic fraction behaved like kerosene, and that the stove oil was apparently unaffected.

A comparison of the odorless-kerosene fractions with the stove oil no. 1 brings out a significant fact concerning the toxicity gained by oil fractions stored for some time. The kerosene, originally almost free of toxicity and injurious only to grasses, gained toxicity to all three plants. The stove-oil fraction, which resembled the kerosene in boiling range, was, both originally and after storage, toxic to flax and barley but nontoxic to carrots. The toxicant that developed in odorless kerosene is therefore nonspecific, whereas that

originally present and unchanged by storage in stove oil is highly specific. Both kerosene and gasoline samples stored in the light gained this same nonspecific toxicity—a fact indicating that a new type of compound resembling in specificity those responsible for chronic toxicity has appeared in the stored samples.

TABLE 36
TOXICITY OF STORED GASOLINE AND KEROSENE TO FLAX, BARLEY, AND CARROTS;
NOVEMBER 6, 1945

Oil fraction and date obtained	Injury to flax (F), barley (B), and carrots (C), in per cent														
	1 day			2 days			3 days			5 days			9 days		
	F	B	C	F	B	C	F	B	C	F	B	C	F	B	C
Kerosene, Nov. 5, 1945	0	0*	0	0	50	0	0	75	0	0	75	0	0§	90	0
Kerosene, May 16, 1945, D 1†	0	0	0	0	50	0	0	65	0	0	75	0	0§	90	0
Kerosene, May 16, 1945, D ½†	0	0	0	0	50	0	0	65	0	0	75	0	0§	90	0
Kerosene, May 16, 1945, L 1†	100	100	70	100	100	70	100	100	70	100	100	60	100	100	50
Kerosene, May 16, 1945, L ½†	75	25	20	75	60	20	75	80	20	60	90	10	60	90	5
Odorless kerosene, Jan. 1, 1944, D 1†	5	0	10	10	20	10	10	40	15	10	60	10	10	90	5
Odorless kerosene, Jan. 1, 1944, L ½†	90	50	20	95	75	25	95	85	25	95	90	15	100	95	5
Range fuel, Nov. 5, 1945	100	100	20	100	100	15	100	100	15	100	100	20	100	100	15
E-1381, April 1, 1945, L	100	90	35	100	100	25	100	100	25	100	100	15	100	100	10
E-1380, April 1, 1945, L	100	100	50	100	100	50	100	100	50	100	100	40	100	100	30
Gas—regular motor fuel, Feb., 1945, L	100	95	65	100	95	65	100	95	65	100	90	50	100	90	35
Shell white gas, Sept., 1943, L	100	100	90	100	100	90	100	100	90	100	100	85	100	100	75
Stove oil no. 1, Sept. 1, 1943, L	100	90	0	100	100	0	100	100	0	100	100	0	100	100	0
Isoparaffin, heavy, April 1, 1944, L	100	25	15	100	75	20	100	95	35	100	100	30	100	100	25

	12 days			16 days			23 days			30 days			34 days		
	F	B	C	F	B	C	F	B	C	F	B	C	F	B	C
Kerosene, Nov. 5, 1945	0	95	0	0	100	0	0	100	0	0	100	0	0	100	0
Kerosene, May 16, 1945, D 1†	0	95	0	0	100	0	0	100	0	0	100	0	0	100	0
Kerosene, May 16, 1945, D ½†	0	95	0	0	100	0	0	100	0	0	100	0	0	100	0
Kerosene, May 16, 1945, L 1†	100	100	40	100	100	30	100	100	15	100	100	10	100	100	10
Kerosene, May 16, 1945, L ½†	50	100	5	40	100	5	25	100	0	25	100	0	25	100	0
Odorless kerosene, Jan. 1, 1944, D 1†	25	95	5	15	100	5	10	100	0	10	100	0	5	100	0
Odorless kerosene, Jan. 1, 1944, L ½†	100	100	5	100	100	5	100	100	0	100	100	0	100	100	0
Range fuel, Nov. 5, 1945	100	100	15	100	100	10	100	100	10	100	100	5	100	100	5
E-1381, April 1, 1945, L	100	100	10	100	100	10	100	100	5	100	100	5	100	100	5
E-1380, April 1, 1945, L	100	100	30	100	100	25	100	100	20	100	100	15	100	100	10
Gas—regular motor fuel, Feb., 1945, L	100	90	30	100	90	25	100	90	20	100	90	15	100	90	10
Shell white gas, Sept., 1943, L	100	100	65	100	100	60	100	90	40	100	100	30	100	100	30
Stove oil no. 1, Sept. 1, 1943, L	100	100	0	100	100	0	100	100	0	100	100	0	100	100	0
Isoparaffin, heavy, April 1, 1944, L	100	100	25	100	100	20	100	100	10	100	100	5	100	100	0

* Barley plants sprayed with fresh kerosene showed positive geotropism 24 hours after spraying. Stored kerosene did not have this effect.

† D signifies storage in the dark: 1, in a full bottle; ½, in a bottle only half full.

‡ L signifies storage in the light.

§ Cotyledons turned yellow and died but there was no permanent injury.

Because of the marked increase in toxicity of the gasoline samples to carrots, tests were run on the lighter three gasoline fractions previously used, on all four stove-oil fractions, and on the mineral seal oil stored both in the dark and in the light. The data appear in table 37. Considering the results on carrots, to which the gasoline was not originally toxic, one sees that storage in

the light had considerably increased the nonspecific acute toxicity of these samples. Stove-oil fractions had not changed. Mineral seal oil, though not toxic to carrots, had increased in toxicity to flax and barley.

TABLE 37

TOXICITY OF GASOLINE AND STOVE-OIL FRACTIONS, STOVE-OIL SAMPLES, AND MINERAL SEAL OIL TO FLAX, BARLEY, AND CARROTS; NOVEMBER 9, 1945

Fraction no. and storage condition	Injury to flax (F), barley (B), and carrots (C), in per cent														
	1 day			2 days			3 days			4 days			6 days		
	F	B	C	F	B	C	F	B	C	F	B	C	F	B	C
Shell white gasoline:															
1 L*.....	100	90	60	100	90	60	100	90	60	100	85	60	100	80	50
2 L.....	100	90	90	100	100	90	100	100	90	100	100	85	100	100	80
3 L.....	100	100	85	100	100	85	100	100	85	100	100	85	100	100	80
Stove oil:															
1 L.....	95	90	0	100	100	5	100	100	5	100	100	5	100	100	5
2 L.....	100	90	0	100	100	0	100	100	0	100	100	0	100	100	0
3 L.....	100	80	0	100	100	0	100	100	0	100	100	0	100	100	0
4 L.....	100	80	5	100	100	5	100	100	5	100	100	5	100	100	5
Sea Side stove oil:															
1 Lt.....	100	90	5	100	100	0	100	100	0	100	100	0	100	100	0
2 Lt.....	100	95	90	100	100	95	100	100	95	100	100	95	100	100	95
Mineral seal oil:															
L.....	0	0	0	0	0	0	0	0	0	0	0†	0	5	0†	0
D.....	0	0	0	0	0	0	0	0	0	0	0†	0	0	0†	0
	9 days			16 days			24 days			31 days			38 days		
	F	B	C	F	B	C	F	B	C	F	B	C	F	B	C
Shell white gasoline:															
1 L*.....	100	75	40	100	50	25	100	20	20	100	15	20	100	0	15
2 L.....	100	100	65	100	100	40	100	100	25	100	100	25	100	100	20
3 L.....	100	100	70	100	100	50	100	100	35	100	100	25	100	100	20
Stove oil:															
1 L.....	100	100	5	100	100	0	100	100	0	100	100	0	100	100	0
2 L.....	100	100	0	100	100	0	100	100	0	100	100	0	100	100	0
3 L.....	100	100	0	100	100	0	100	100	0	100	100	0	100	100	0
4 L.....	100	100	5	100	100	0	100	100	0	100	100	0	100	100	0
Sea Side stove oil:															
1 Lt.....	100	100	0	100	100	0	100	100	0	100	100	0	100	100	0
2 Lt.....	100	100	90	100	100	80	100	100	80	100	100	65	100	100	50
Mineral seal oil:															
L.....	15	5†	0	25	25	0	50	50	0	50	75	0	40	80	0
D.....	3	0†	0	10	15	0	50	40	0	60	70	0	50	60	0

* The designation L indicates that the sample had been stored in the light; D indicates storage in the dark.

† Sea Side stove oil no. 1 had a gravity rating (°A.P.I.) of 33.7; no. 2 of 34.7.

‡ Four days after spraying, the barley plants showed strong positive geotropism. This lasted for somewhat over a week.

A possible explanation for these observations is that the changes occurring in low-boiling refined petroleum fractions in the light involve formation of peroxides of the aliphatic compounds. Peroxide production may be autocatalytic; the time of storage may simply represent the induction period necessary to bring about rapid oxidation in the air; light greatly accelerates

this process. When such a stored oil is applied to the plant, rapid formation of peroxide causes enhanced acute, nonselective toxicity. Heavier fractions less subject to peroxide formation may undergo similar changes on a reduced scale and at a much slower rate. The aromatic compounds present in unrefined fractions act as antioxidants; but, being toxic, their original toxicity would mask peroxide effects. From these postulations it follows that the lighter the fraction the greater is peroxide formation; the more highly refined, the greater the relative increase in toxicity; the higher the toxicity due to peroxides, the lower the selectivity.

PURE COMPOUNDS

There are two approaches to the experimental determination of the toxic nature of oils. One is to use oil fractions of different types and to explain (if possible) their behavior on the basis of existing information regarding their composition. Such an approach has been used in the work described in the preceding sections.

Toxicity of Aromatic Compounds. A second approach is to use pure¹⁰ compounds of known structure and to compare the results with those obtained with the oil fractions. Since the work on oil fractions had indicated that aromatic compounds play an important role, the first test compared the toxicity of benzene, toluene, and xylene on grass. Cultures were sprayed with the pure hydrocarbons and with mixtures containing 50 per cent, 25 per cent, and 12.5 per cent of the hydrocarbons in odorless kerosene. Table 38 presents the results.

The toxicity of these three compounds evidently increases with increasing substitutions in the benzene ring, the xylene being the most toxic. Their boiling points are as follows: benzene, 176.7° F; toluene, 231.8° F; and xylene, 280.4° to 287.6° F. Xylene was much lower in toxicity than the compounds responsible for herbicidal action in oil fractions: it was required in full strength to injure the grass seriously, and at concentrations below 50 per cent it was nontoxic.

For a clearer picture of the comparative toxicity of xylene, the experiment reported in table 39 was performed. Here xylene, TS-28 solvent, gasoline, and stove oil were used in varying volumes on grass. Judging from the results, xylene is approximately equal to gasoline in toxicity but is less toxic than TS-28 and stove oil. These latter both contain aromatics having higher boiling points than xylene. Gasoline and stove oil have only about 20 to 30 per cent unsaturates, however, whereas xylene and TS-28 solvent are both practically pure aromatics.

To study the relation of xylene-type aromatics to selectivity of oils, xylene was applied to grass and carrot cultures in a range of concentrations, the heavy isoparaffinic fraction being used as a diluent. Cracked gasoline was used in comparison as a check on the toxicity of an oil fraction. The results are reported in table 40.

¹⁰ The hydrocarbons used in these studies were not tested for purity. They were obtained from commercial supply houses or from interested industrial firms and were presumed to be reasonably pure.

TABLE 38
TOXICITY OF BENZENE, TOLUENE, AND XYLENE TO GRASS;
APRIL 4, 1944

Hydrocarbon and amount of hydrocarbon (H) and diluent (odorless kerosene) applied per culture	Injury to grass, in per cent		
	1 day	2 days	3 days
Benzene:			
6 ml H, 0 ml OK.....	50	60	60
3 ml H, 3 ml OK.....	5	10	10
1½ ml H, 4½ ml OK.....	0	5	5
¾ ml H, 5¾ ml OK.....	0	0	0
Toluene:			
6 ml H, 0 ml OK.....	75	80	80
3 ml H, 3 ml OK.....	25	25	25
1½ ml H, 4½ ml OK.....	0	5	5
¾ ml H, 5¾ ml OK.....	0	0	0
Xylene:			
6 ml H, 0 ml OK.....	95	95	95
3 ml H, 3 ml OK.....	50	50	50
1½ ml H, 4½ ml OK.....	0	0	0
¾ ml H, 5¾ ml OK.....	0	0	0

TABLE 39
TOXICITY OF XYLENE, TS-28 SOLVENT, WHITE GASOLINE, AND STOVE OIL TO FOXTAIL GRASS; APRIL 12, 1944

Oil and amount applied per culture	Injury to grass, in per cent					
	1 day	2 days	3 days	7 days	12 days	17 days
Xylene:						
10.....	0	20	20	25	30	50
15.....	60	70	80	80	80	80
22.....	80	90	90	90	90	90
32.....	95	95	95	95	95	95
TS-28 solvent:						
10.....	25	30	35	50	65	70
15.....	80	80	90	95	100	100
22.....	90	90	95	95	95	100
32.....	95	95	100	100	100	100
White gasoline:						
10.....	15	15	20	20	25	40
15.....	75	75	80	80	80	90
22.....	80	80	80	85	85	85
32.....	90	90	90	90	90	90
Stove oil:						
10.....	40	40	40	65	75	75
15.....	60	60	65	90	95	95
22.....	80	80	85	98	100	100
32.....	95	95	95	100	100	100

The data show that with these oils, as with other herbicides, selectivity is only relative (Crafts, 1946). Xylene, if concentrated enough, will kill carrots as well as grass. Mixed 50:50 with the relatively nontoxic isoparaffinic fraction, it was highly selective, damaging young grass 90 per cent but causing carrots only a slight injury, from which they soon recovered.

TABLE 40
TOXICITY OF XYLENE AND GASOLINE TO GRASS AND CARROTS; JUNE 20, 1944

Oil and amounts of oil (O) and diluent (isoparaffin) applied per culture	Injury to grass (G) and carrots (C), in per cent											
	1 day		2 days		3 days		4 days		5 days		8 days	
	G	C	G	C	G	C	G	C	G	C	G	C
Xylene:												
0 ml O, 8 ml IP.....	0	0	0	0	0	0	0	0	0	0	0	0
1 ml O, 7 ml IP.....	0	0	0	0	0	0	0	0	0	0	0*	0
2 ml O, 6 ml IP.....	10	0	10	0	10	0	10	0	0	0	0*	0
3 ml O, 5 ml IP.....	25	0	35	0	35	0	35	0	35	0	40	0
4 ml O, 4 ml IP.....	90	10	90	10	90	10	90	10	90	5	90	5
5 ml O, 3 ml IP.....	100	50	90	75	90	60	90	60	90	50	90	75
6 ml O, 2 ml IP.....	100	100	100	100	100	100	100	100	100	100	100	100
7 ml O, 1 ml IP.....	100	100	100	100	100	100	100	90	100	100	90	100
8 ml O, 0 ml IP.....	100	100	100	100	90	100	80	100	80	100	80	100
Cracked gasoline:												
8 ml O, 0 ml IP.....	90	10	100	20	100	25	100	25	95	15	95	10
6 ml O, 2 ml IP.....	50	0	75	0	75	0	75	0	75	0	80	0
4 ml O, 4 ml IP.....	20	0	50	0	50	0	60	0	60	0	75	0

* Chronic injury starting.

TABLE 41
TOXICITY OF BENZENE, ETHYLBENZENE, AND DIETHYLBENZENE TO FLAX, BARLEY,
AND CARROTS; JANUARY 15, 1946

Oil and amounts of oil (O) and diluent (isoparaffin) applied per culture	Injury to flax (F), barley (B), and carrots (C), in per cent								
	1 day			2 days			3 days		
	F	B	C	F	B	C	F	B	C
Benzene:									
8 ml O, 0 ml IP.....	75	50	40	90	50	35	90	50	30
6 ml O, 2 ml IP.....	50	5	5	60	25	5	60	25	10
4 ml O, 4 ml IP.....	50	5	0	60	5	5	40	5	5
2 ml O, 6 ml IP.....	25	0	0	40	5	0	20	0	0
Ethylbenzene:									
8 ml O, 0 ml IP.....	95	90	90	100	100	95	100	100	100
6 ml O, 2 ml IP.....	90	90	80	100	100	90	100	100	90
4 ml O, 4 ml IP.....	50	50	40	90	90	75	90	90	75
2 ml O, 6 ml IP.....	10	10	0	60	75	5	70	75	10
Diethylbenzene:									
8 ml O, 0 ml IP.....	95	90	95	100	100	100	100	100	100
6 ml O, 2 ml IP.....	90	90	80	100	100	100	100	100	100
4 ml O, 4 ml IP.....	50	50	10	100	100	30	100	100	40
2 ml O, 6 ml IP.....	10	10	0	90	90	5	95	90	5

Cracked gasoline when used straight caused some injury to carrots. This contrasts with the tests reported in table 4, in which a sample of commercial white gasoline, probably a blend of cracked and straight-run gasolines, proved nontoxic to carrots. At 75 per cent in isoparaffin, cracked gasoline was not injurious to them and did not completely kill the grass plants.

These experiments indicate how critical is the problem of providing a selective oil spray suitable for commercial application under varying field conditions. The fact that stove oil, a low-priced commercial fuel fraction, has proved so effective is purely fortuitous. By coincidence this fraction proved to combine the particular properties required for killing weeds in carrots. As al-

TABLE 42
TOXICITY OF PURE AROMATIC HYDROCARBONS* TO BARLEY, CARROTS, AND ONIONS;
MAY 5, 1944

Compound (mixture of 20 per cent concentration in isoparaffin)	Injury to barley (B), carrots (C), and onions (O), in per cent											
	1 day			3 days			5 days			7 days		
	B	C	O	B	C	O	B	C	O	B	C	O
Isopropylbenzene.....	0	0	0	0	0	0	0	0	0	5	0	0
Diisopropylbenzene.....	0	0	0	0	0	0	0	0	0	10	5	0
Triisopropylbenzene.....	0	0	0	0	0	0	5	0	0	15	5	0
Tetraisopropylbenzene.....	0	0	0	0	0	0	5	0	0	20	10	0
Cymene.....	5	0	0	5	0	0	5	0	0	5	0	0
Methylcyclopentane.....	0	0	0	0	0	0	0	0	0	5	0	0
	10 days			13 days			15 days			18 days		
	B	C	O	B	C	O	B	C	O	B	C	O
Isopropylbenzene.....	10	0	0	20	0	0	30	0	0	50	0	0
Diisopropylbenzene.....	20	5	0	30	5	0	60	5	0	70	5	0
Triisopropylbenzene.....	50	10	0	80	15	0	95	15	0	100	15	0
Tetraisopropylbenzene.....	40	15	0	50	20	0	60	20	0	80	20	0
Cymene.....	10	10	0	15	15	0	25	15	0	40	15	0
Methylcyclopentane.....	10	10	0	20	10	0	40	10	0	40	10	0

* The pure aliphatic compounds *n*-hexane, neohexane, isooctane, and *n*-cetane, tested at the same time, produced no toxicity within the 18-day observation period.

ready indicated, however, this fraction is just on the limit with respect to its gravity rating; a lighter fraction would be more selective and hence safer to use from the herbicidal standpoint. But the fact that it would also be more volatile, and hence more inflammable, should not be overlooked.

Toxicity of Benzenes. Table 41 shows the results of spraying flax, barley, and carrot cultures with benzene, ethylbenzene, and diethylbenzene, used pure or diluted with the heavy isoparaffinic fraction. The toxicity seems to increase with increased substitution of ethyl groups through this series. Except at the lowest concentrations, these compounds show little selectivity.

The next tests involved a series of higher-boiling-point aromatic hydrocarbons. Pure samples of iso-, diiso-, triiso-, and tetraisopropylbenzene, mixed with the isoparaffinic diluent, were sprayed on barley, onions, and carrots. Included in the experiment also were cymene and methylcyclopentane. *n*-Hexane, neohexane, isooctane, and *n*-cetane were used without dilution; being aliphatic hydrocarbons, they offer promise as nontoxic diluents.

Table 42 presents the results of the tests. Among the substituted benzenes there was an increase in toxicity with increase in isopropyl substitutions up to

the triisopropyl compound and then a small decrease with the tetraisopropyl compound. All toxicity was chronic, and the cymene and methylcyclopentane samples also showed toxicity. Because the isoparaffin used as a diluent in these tests also exhibits some chronic toxicity, the absolute level of toxicity is somewhat in doubt. The differences are significant because the concentrations are the same: table 42 reports results with 20 per cent concentrations of these toxicants. The experiments included 10 per cent concentrations as well, and these showed some toxicity. Such results contrast with those of tables 38 and 40, where concentrations of toxicants in the range of 25 per cent

TABLE 43
TOXICITY OF ISOPROPYL AND DIISOPROPYLBENZENES TO BARLEY;
JUNE 22, 1944

Oil and amounts of oil (O) and diluent (isoparaffin) applied per culture	Injury to barley, in per cent				
	1 day	2 days	3 days	4 days	6 days
Isopropylbenzene					
10 ml O, 0 ml IP	95	95	95	95	98
8 ml O, 2 ml IP	80	80	80	80	85
6 ml O, 4 ml IP	70	70	70	70	80
4 ml O, 6 ml IP	50	60	60	70	80
2 ml O, 8 ml IP	0	10	10	10	15
0 ml O, 10 ml IP	0	0	0	0	0
Diisopropylbenzene					
10 ml O, 0 ml IP	100	100	100	100	100
8 ml O, 2 ml IP	100	100	100	100	100
6 ml O, 4 ml IP	95	95	97	97	100
4 ml O, 6 ml IP	50	70	80	85	95
2 ml O, 8 ml IP	10	15	15	20	25
0 ml O, 10 ml IP	0	0	0	0	0

to 0 per cent showed no toxicity. Apparently the toxicants in table 42 more nearly resemble those of petroleum fractions in effectiveness than do benzene, toluene, and xylene.

The four chemically pure aliphatic hydrocarbons were nontoxic throughout the 18-day period of observation. The hexanes were so volatile that when applied with the atomizer they did not even wet the plants. The octane was scarcely more effective. The *n*-cetane wet the plants thoroughly, and they appeared oil-soaked for over a week; but still there were no injury symptoms beyond a stunting of the plants. Later experiments, carried on for longer periods proved that even *n*-cetane is toxic to grass species. Evidently, chemically pure samples of these aliphatic hydrocarbons are the safest diluents available since even highly refined samples of petroleum fractions have proved more toxic.

Methylcyclopentane is a saturated ring compound, and one might presume that it would not be toxic. The test, however, proved that it was of about the same order of toxicity to carrots as triisopropylbenzene and almost as toxic to barley as isopropylbenzene. Cymene was about as toxic as methylcyclopentane. Lack of toxicity of all these compounds to the onion cultures resulted from the fact that the plants were larger than those used in previous tests. As onions grow older, the leaves become tougher and more resistant to oil tox-

icity, particularly as growth slows down and the plants approach maturity. The onions used in these tests had practically ceased to grow. The barley and carrot cultures were also relatively mature.

The next test on pure compounds involved a comparison of iso- and diisopropylbenzenes in concentration series, with isoparaffin as a diluent. The tri- and tetraisopropylbenzenes were not available at the time in sufficient quantities for this test. Table 43 presents the data.

TABLE 44

TOXICITY TESTS OF AROMATIC AND CYCLIC HYDROCARBONS ON BROAD-LEAVED WEEDS AND GRASS; OCTOBER 30, 1944

Concentration and compound	Injury to broad-leaved weeds (BW) and grass (G), in per cent											
	1 day		2 days		3 days		4 days		5 days		6 days	
	BW	G	BW	G	BW	G	BW	G	BW	G	BW	G
20 per cent concentration in isoparaffin:												
Isopropylbenzene.....	20	0	25	0	20	5	20	5	25	10	25	25
Diisopropylbenzene.....	30	0	50	5	60	10	70	15	80	20	80	60
Triisopropylbenzene.....	5	0	0	0	0	0	0	0	0	25	10	75
Tetraisopropylbenzene.....	40	10	60	25	70	25	75	30	85	40	85	75
Methylcyclopentane.....	5	0	10	0	10	0	10	0	10	5	10	15
Cyclohexane.....	20	0	75	40	85	40	90	50	90	65	90	75
Methylcyclohexane.....	20	0	25	5	25	5	25	10	25	15	25	25
Cymene.....	2	0	5	0	10	5	15	5	20	10	20	15
10 per cent concentration in isoparaffin:												
Isopropylbenzene.....	5	0	5	0	5	0	5	0	5	5	5	15
Diisopropylbenzene.....	10	0	10	0	10	0	15	0	15	5	15	15
Triisopropylbenzene.....	2	0	0	0	0	0	0	0	0	10	5	50
Tetraisopropylbenzene.....	20	5	20	5	20	10	20	15	40	20	40	75
Methylcyclopentane.....	2	0	5	0	5	0	5	0	10	5	10	15
Cyclohexane.....	20	0	40	5	40	10	60	20	70	30	70	50
Methylcyclohexane.....	10	0	15	0	15	0	15	0	15	5	15	10
Cymene.....	0	0	2	0	2	0	2	0	5	5	5	15

These results confirm the implication that toxicity increases with additional substitutions, the diisopropyl compound proving the more toxic. Since the barley plants were young and succulent, acute toxicity was much more in evidence. The less concentrated mixtures became appreciably more toxic, however, during the 6-day observation period; and had the tests been continued longer, toxicity would undoubtedly have increased even further.

Aromatic and Cyclic Hydrocarbons. In one additional test conducted on these compounds, the four isopropyl-substituted benzenes and methylcyclopentane, cyclohexane, methylcyclohexane, and cymene were compared, all at 10 and 20 per cent concentrations in isoparaffin. Table 44 gives the data on these tests.

Judging from the results of this experiment, toxicity of the substituted benzenes increases with increasing number of substitutions, but not in a simple series; the mono- and tri-substitutions are low in toxicity; the di- and tetra-substitutions are high. Since the limited samples supplied were used up in these tests and since isoparaffin had to be used as a diluent, no further testing was done with them.

Cyclohexane proved more toxic than methylcyclohexane; methylcyclopentane and cymene again were comparable in toxicity and below the 6-carbon ring compounds mentioned above.

TABLE 45
TOXICITY TESTS WITH AROMATIC HYDROCARBONS ON BROAD-LEAVED WEEDS
AND GRASS; OCTOBER 30, 1944

Concentration and compound	Injury to broad-leaved weeds (BW) and grass (G), in per cent											
	1 day		2 days		3 days		4 days		6 days		8 days	
	BW	G	BW	G	BW	G	BW	G	BW	G	BW	G
40 per cent concentration in isoparaflin:												
Benzene	5	0	5	0	5	0	5	0	5	5	5	15
Toluene	0	0	0	0	0	0	0	0	0	0	0	5
Ethylbenzene	10	0	10	0	20	0	20	0	20	5	20	25
Xylene	20	5	10	0	10	0	15	0	15	5	15	15
20 per cent concentration in isoparaflin:												
Benzene	0	0	2	0	2	0	2	0	2	2	2	15
Toluene	0	0	0	0	0	0	0	0	0	0	0	15
Ethylbenzene	5	0	5	0	5	0	5	0	5	5	5	15
Xylene	0	0	2	0	2	0	2	0	2	0	2	15
10 per cent concentration in isoparaflin:												
Benzene	0	0	0	0	0	0	0	0	0	2	0	15
Toluene	0	0	0	0	0	0	0	0	0	0	0	15
Ethylbenzene	0	0	0	0	0	0	0	0	5	5	5	15
Xylene	0	0	0	0	0	0	0	0	0	0	0	15

TABLE 46
TOXICITY OF CYCLOHEXANE AND CYCLOHEXENE TO FLAX, BARLEY, AND CARROTS;
JANUARY 15, 1946

Oil and amounts of oil (O) and diluent (isoparaflin) applied per culture	Injury to flax (F), barley (B), and carrots (C), in per cent								
	1 day			2 days			3 days		
	F	B	C	F	B	C	F	B	C
Cyclohexane:									
8 ml O, 8 ml IP	75	40	20	60	30	20	40	25	20
6 ml O, 2 ml IP	10	0	5	10	5	5	10	10	5
4 ml O, 4 ml IP	5	0	5	5	15	0	5	25	10
2 ml O, 8 ml IP	5	0	0	15	20	0	40	60	10
Cyclohexene:									
8 ml O, 0 ml IP	95	70	40	90	70	40	90	70	40
8 ml O, 2 ml IP	90	50	20	90	65	20	90	80	25
4 ml O, 4 ml IP	50	15	0	50	80	10	80	90	15
2 ml O, 6 ml IP	10	0	0	75	90	15	80	90	15

Table 45, presenting tests run simultaneously with those of table 44, shows that ethylbenzene is comparable with xylene in toxicity. Their boiling points are nearly the same, the former being a bit lighter. The pure aliphatic hydrocarbons, n -hexane, neohexane, isooctane, and n -cetane proved nontoxic on these rather mature plants; apparently some oils may be used on certain plants without causing injury.

Table 46 presents data on tests comparing cyclohexane and cyclohexene in pure and in concentration series, with isoparaffin as a diluent. The results show the cyclohexene to be the more toxic, the carrots being somewhat less susceptible to both compounds than were flax and barley. The heavy isoparaffinic fraction used as a diluent in this experiment had aged in a clear glass bottle and was itself somewhat toxic.

Mercaptans. The sulfur compounds present in most crude oils often constitute a problem because they are undesirable in refined products. They include the mercaptans, a group of foul-smelling liquids that might offer herbicidal possibilities. Samples of *n*-propyl, isopropyl, *n*-butyl, and isobutyl mercaptans have been tested. Used pure, these compounds caused almost

TABLE 47

TOXICITY OF SOME MERCAPTANS TO BARLEY, FOXTAIL GRASS, MUSTARD, CARROTS, AND FLAX; MARCH 8, 1945

Mercaptan	Injury to barley (B), foxtail grass (Fx), mustard (M), carrots (C), and flax (F), in per cent																			
	6 hours					1 day					4 days					6 days				
	B	Fx	M	C	F	B	Fx	M	C	F	B	Fx	M	C	F	B	Fx	M	C	F
<i>n</i> -Propyl.....	5	15	35	10	15	5	10	40	10	20	5	10	35	0	20	0	5	30	0	20
Isopropyl.....	0	0	15	0	5	0	0	20	0	5	0	0	10	0	5	0	0	10	0	5
<i>n</i> -Butyl.....	5	25	50	10	25	5	20	30	15	20	5	15	35	5	20	5	15	25	0	15
Isobutyl.....	5	2	20	15	2	5	0	40	15	0	0	0	30	5	0	0	0	35	0	0

immediate injury to leaves. They quickly evaporated, however, and little injury occurred after they had left the leaves of the test plants.

Table 47 presents the results. The normal compounds were somewhat more toxic than the branched-chain compounds. There were no significant differences between propyl and butyl compounds. None of these compounds was toxic enough to offer any promise as a herbicide. In 50:50 dilutions with isoparaffin, almost no toxicity was shown.

Tests on Chain and Cyclic Hydrocarbons. Since the odorless kerosene and the isoparaffinic fractions tested as nontoxic diluents had both proved toxic in experiments covering several days (p. 99), a test was conducted using *n*-cetane, hexane, and samples of the unsulfonated residues of stove oil and of isoparaffin that had been stored in the light. Cyclohexene was included as a toxic compound for comparison. The test ran over 5 weeks. Table 48 presents the results.

Cyclohexene is a very light hydrocarbon. It severely damaged all three crops and killed the flax completely. The effects on barley and carrots were not permanent. *n*-Hexane also caused some acute injury, from which all plants recovered.

Of the unsulfonated residues, that of the isoparaffin severely damaged flax and barley but proved nontoxic to carrots. The stove-oil residue, in contrast, lacked acute toxicity; but the barley manifested chronic injury and finally died.

n-Cetane killed none of the plants. After about 2 weeks, however, the flax leaves began to show deformation at the tips, and the barley was stunted and tillered more heavily than did unsprayed checks. These effects persisted, the flax plants having their tip leaves clustered and folded as shown in figure 3, *B* (p. 97). But the growing points were not killed; and by the end of the experiment the injured region, visible as a whorl of distorted leaves, had been left behind as normal growth proceeded.

TABLE 48

TOXICITY OF *n*-CETANE, *n*-HEXANE, CYCLOHEXENE, AND THE UNSULFONATED RESIDUES OF STOVE OIL AND HEAVY ISOPARAFFIN TO FLAX, BARLEY, AND CARROTS;
NOVEMBER 9, 1945

Compound	Injury to flax (F), barley (B), and carrots (C), in per cent																	
	1 day			2 days			3 days			6 days			9 days			13 days		
	F	B	C	F	B	C	F	B	C	F	B	C	F	B	C	F	B	C
<i>n</i> -Cetane.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>n</i> -Hexane.....	15	20	0	10	20	0	10	20	0	10	20	0	10	15	0	5	10	0
Cyclohexene.....	100	50	50	100	50	50	100	50	50	100	40	40	100	35	40	100	25	30
Stove oil, UR.....	0	0	0	0	20	0	0	30	0	0	40	0	0	60	0	0	80	0
Isoparaffin, UR.....	75	15	0	90	40	0	90	60	0	90	70	0	90	70	0	90	70	0
	16 days			20 days			24 days			27 days			31 days			38 days		
	F	B	C	F	B	C	F	B	C	F	B	C	F	B	C	F	B	C
<i>n</i> -Cetane.....	0	0	0	0*	0†	0	0*	0†	0	0*	0†	0	0*	0†	0	0*	0†	0
<i>n</i> -Hexane.....	5	10	0	5	10	0	5	10	0	0	5	0	0	0	0	0	0	0
Cyclohexene.....	100	25	25	100	25	25	100	15	15	100	10	10	100	0	5	100	0	0
Stove oil, UR.....	0	90	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0
Isoparaffin, UR.....	90	75	0	90	75	0	90	80	0	90	85	0	85	85	0	85	95	0

* Tip leaves deformed.

† Plants oil-soaked, stunted, and severely infected with powdery mildew.

The cetane-sprayed barley plants continued to be stunted. They appeared dark green and oil-soaked; during the final 2 weeks, they became heavily infected with mildew. Judging from these results, no diluent so far tested has been completely satisfactory. For short-time tests on fairly mature plants (see table 45) *n*-hexane, isooctane, and *n*-cetane proved nontoxic. On young succulent plants of susceptible species, however (table 48), these compounds appear toxic, the lighter ones producing acute toxicity, the heavier a slow-developing chronic injury. At present no completely nontoxic oil is known that can be used under a wide range of conditions as a diluent for oil-spray experiments. Even unsulfonated residues of relatively nontoxic isoparaffin develop toxicity if stored in the light.

SELECTIVITY OF OILS

Because this study was initiated in response to questions on the specificity of oils, and because most tests were run with two or more species of test plants, some evidence of selectivity is to be found in almost every table. Some that

warrant reinspection are 3, 4, 5, 6, 8, 10, 15, 17, 20, 21, 23, 28, 29, 30, 31, 32, 34, 35, 36, 37, 40, 41, 42, 46, 47, and 48.

Type of Compound. Considering, first, the type of compound responsible for selectivity, table 40 (p. 122) shows that xylene will severely injure grass at a concentration that scarcely harms carrots. Table 49 gives confirming evidence over a more extended observation period. Table 50 indicates that onions and flax may also tolerate low concentrations of aromatics.

TABLE 49

TOXICITY OF XYLENE AND ISOPARAFFIN MIXTURES TO CARROTS, MUSTARD,
AND FOXTAIL GRASS; MARCH 14, 1945

Amounts of xylene and diluent (isoparaffin) applied per culture	Injury to carrots (C), mustard (M), and foxtail grass (Fx), in per cent											
	1 day			2 days			3 days			4 days		
	C	M	Fx	C	M	Fx	C	M	Fx	C	M	Fx
10 ml X, 0 ml IP.....	95	100	100	100	100	100	100	100	100	100	100	100
8 ml X, 2 ml IP.....	90	95	100	95	100	100	95	100	100	95	100	100
6 ml X, 4 ml IP....	80	95	95	85	95	95	85	100	98	85	100	98
5 ml X, 5 ml IP.....	20	95	90	25	95	95	35	95	98	40	95	98
4 ml X, 6 ml IP.....	5	95	75	5	95	80	5	95	90	5	95	95
0 ml X, 10 ml IP.....	0	0	0	0	0	0	0	0	0	0	0	0
	6 days			8 days			13 days			18 days		
	C	M	Fx	C	M	Fx	C	M	Fx	C	M	Fx
10 ml X, 0 ml IP.....	100	100	100	100	100	100	100	100	98	100	100	98
8 ml X, 2 ml IP.....	90	100	100	90	100	100	80	100	98	90	100	99
6 ml X, 4 ml IP.....	75	100	95	75	100	95	70	100	98	50	100	98
5 ml X, 5 ml IP.....	40	95	100	40	100	100	30	98	100	15	98	100
4 ml X, 6 ml IP.....	5	98	95	5	98	98	0	98	98	0	95	99
0 ml X, 10 ml IP.....	0	0	0	0	0	5	0	0	10	0	0	10

Table 42 (p. 123) shows that the isopropyl-substituted benzenes are somewhat selective, but that they injure carrots more than do such petroleum fractions as gasoline and stove oil. Toxicity of the isopropylbenzenes is chronic and results with Diesel oil (compare table 4, p. 87) show that the chronic toxicants exhibit less selectivity between weeds and carrots than do compounds causing acute toxicity.

There is apparently a relation between boiling point of a toxicant, type of toxicity, and selectivity. Table 10 (p. 98) compares two kerosenes and mineral seal oil. The latter appeared relatively nontoxic for the first week; but plants gradually developed injury until, after 24 days, all were severely affected. A significant fact is that carrots were damaged more than grass. Tests on carrots with the fuel oil used in the tests on grasses reported in table 12 (p. 99) proved that this heavy crude fraction has similar chronic toxic properties.

As shown by table 10 (p. 98), when kerosene was applied the grass was dead after 24 days, whereas broad-leaved weeds, carrots, and flax were unharmed. This result, which has been obtained many times, indicates a possible

TABLE 50

TOXICITY OF XYLENE, AND XYLENE AND ODORLESS KEROSENE MIXTURES TO FLAX, ONIONS, CARROTS, GRASSES, AND BROAD-LEAVED WEEDS; APRIL 12, 1944

Amounts of xylene and diluent (odorless kerosene) applied per culture	Injury to flax (F), onions (O), carrots (C), grasses (G), and broad-leaved weeds (BW), in per cent														
	1 day					2 days					3 days				
	F	O	C	G	BW	F	O	C	G	BW	F	O	C	G	BW
10 ml X, 0 ml OK	100	50	90	75	90	100	50	90	90	90	100	50	95	90	90
7.5 ml X, 2.5 ml OK	90	50	40	90	90	90	50	80	90	95	95	50	75	95	95
5.0 ml X, 5.0 ml OK	75	20	0	90	75	75	20	0	90	80	75	20	0	95	90
2.5 ml X, 7.5 ml OK	0	0	0	25	10	0	0	0	25	20	0	0	0	25	25
	7 days					12 days					17 days				
	F	O	C	G	BW	F	O	C	G	BW	F	O	C	G	BW
10 ml X, 0 ml OK . .	100	40	100	100	100	100	20	100	100	100	100	10	100	100	100
7.5 ml X, 2.5 ml OK.	100	50	90	100	100	100	50	90	100	100	100	50	90	100	100
5.0 ml X, 5.0 ml OK.	75	40	0	100	100	60	30	0	100	100	60	50	0	100	100
2.5 ml X, 7.5 ml OK.	0	0	0	50	25	0	0	0	75	25	0	0	0	75	25

TABLE 51

TOXICITY OF GASOLINE, STOVE OIL, AND AVON WEED KILLER IN DIFFERENT CONCENTRATIONS TO ONIONS, GRASS, AND WEEDS; FEBRUARY 21, 1944

Oil fraction and amounts of fraction (F) and diluent (odorless kerosene) applied per culture	Injury to onions (O), grass (G), and weeds (W), in per cent											
	2 days			3 days			4 days			5 days		
	O	G	W	O	G	W	O	G	W	O	G	W
Gasoline:												
10 ml F, 0 ml OK	60	90	75	60	95	90	60	99	90	50	100	90
5 ml F, 5 ml OK . .	0	0	25	0	0	25	0	0	25	0	0	25
4 ml F, 6 ml OK . . .	0	0	10	0	0	10	0	0	10	0	0	10
3 ml F, 7 ml OK . . .	0	0	5	0	0	5	0	0	5	0	0	5
2 ml F, 8 ml OK . . .	0	0	0	0	0	0	0	0	0	0	0	0
Stove oil:												
10 ml F, 0 ml OK...	50	75	90	60	85	90	65	95	90	50	95	95
5 ml F, 5 ml OK . . .	0	50	75	5	60	75	10	60	75	10	50	75
4 ml F, 6 ml OK . . .	0	20	50	0	40	60	5	40	60	5	40	60
3 ml F, 7 ml OK . . .	0	5	25	0	5	40	0	5	30	0	5	25
2 ml F, 8 ml OK . . .	0	0	10	0	0	15	0	0	15	0	0	15
Avon Weed Killer:												
10 ml F, 0 ml OK	100	100	100	100	100	100	100	100	100	100	100	100
5 ml F, 5 ml OK	90	95	100	95	100	100	100	100	100	100	100	100
4 ml F, 6 ml OK	80	90	95	75	100	100	100	100	100	100	100	100
3 ml F, 7 ml OK	50	75	90	50	100	100	50	100	100	50	100	100
2 ml F, 8 ml OK	0	20	30	10	50	75	15	25	80	10	50	80

commercial application because wild oats, foxtail, and other grass species are serious pests in flax. Observations on many oil-spray results indicate that oils in the medium gravity range are specific against grasses. Even when used at concentrations that do not kill the grass plants, oils stunt these weeds and allow the crop to compete more successfully for light and moisture.

Experiments with heavy olefinic and aromatic fractions extracted from petroleum show these compounds to be selective between carrots and weeds (table 28, p. 110). Furthermore, these compounds are fatal to weeds at concentrations of 30 per cent and below. In this characteristic they resemble the naturally occurring selective toxicants of oils much more than do xylene and similar low-boiling simple aromatics.

TABLE 52
TOXICITY OF GASOLINE AND STOVE OIL TO ONIONS, WEEDS, AND GRASS;
FEBRUARY 25, 1944

Oil fraction and amounts applied per culture	Injury to onions (O), weeds (W), and grass (G), in per cent											
	1 day			3 days			5 days			10 days		
	O	W	G	O	W	G	O	W	G	O	W	G
Gasoline:												
7½ ml.	20	95	90	20	100	90	20	100	100	10	100	100
10 ml.	50	100	95	40	100	98	40	100	100	20	100	100
12½ ml.	50	100	95	40	100	100	40	100	100	20	100	100
15 ml.	70	100	100	60	100	100	60	100	100	60	100	100
Stove oil:												
5 ml.	10	50	90	10	50	90	10	90	90	0	100	100
7½ ml.	10	80	90	10	90	100	15	95	100	5	100	100
10 ml.	40	100	75	40	100	90	40	100	100	20	100	100
12½ ml.	50	75	90	40	75	100	60	75	100	40	90	100

According to table 28, the heavy aromatic fraction damaged foxtail grass slightly more than it did flax, a result that confirms the observations in table 10.

Selective Action on Onions. Table 51 compares the toxicities of gasoline, stove oil, and Avon Weed Killer at different concentrations with respect to onions, grass, and broad-leaved weeds. Judging from the results, stove oil has a selective action on weeds in onions. The dilutions of gasoline did not include concentrations between 100 per cent and 50 per cent. Other experiments show that at intermediate concentrations gasoline, too, is selective on onions.

These oils were used in sufficient volume to wet the plants thoroughly. A possible alternative approach to the problem was to apply the fractions in varying volumes, omitting the diluent. Table 52 gives the results of such an experiment. Evidently gasoline and stove oil both act selectively on weeds in onions.

Next, in a study of the possible relation between boiling point and selectivity, the experiments reported in table 53 were performed. These were some of the same fractions reported in table 5 (p. 92). Evidently the heavier fractions are more toxic to onions than are the light ones. The results, although

less consistent than one might wish, do show that grass may be severely injured without harm to young onions and that injury to onions is greatest toward the heavy end. This indication is confirmed by results of field trials. By means of a knapsack sprayer, onions in the field were treated with gasoline, stove oil, and Diesel oil. These oils killed grasses and most broad-leaved weeds. Injury to the onions was negligible from gasoline, not bad from stove oil, but serious from Diesel oil (Crafts and Raynor, 1944).

TABLE 53

TOXICITY OF FRACTIONAL DISTILLATES OF WHITE GASOLINE AND STOVE OIL TO
ONIONS AND GRASSES; MAY 5, 1944

Oil, fraction no. and amounts of fraction (F) and diluent (isoparaffin) applied per culture	Injury to onions (O) and grasses (G), in per cent									
	1 day		3 days		5 days		10 days		15 days	
	O	G	O	G	O	G	O	G	O	G
White gasoline no. 1:										
12 ml F, 0 ml IP..	20	90	10	90	5	90	0	90	0	95
6 ml F, 6 ml IP..	0	10	0	10	0	10	0	20	0	25
White gasoline no. 2:										
12 ml F, 0 ml IP..	30	90	20	90	10	95	0	100	0	100
6 ml F, 6 ml IP..	0	10	0	15	0	15	0	25	0	40
White gasoline no. 3:										
12 ml F, 0 ml IP..	50	95	50	100	40	100	40	100	50	100
6 ml F, 6 ml IP..	10	50	10	60	5	75	0	90	0	90
White gasoline no. 4:										
12 ml F, 0 ml IP..	65	95	60	100	50	100	30	100	20	100
6 ml F, 6 ml IP..	15	75	15	85	10	90	0	90	0	90
Stove oil no. 1:										
12 ml F, 0 ml IP..	70	90	60	90	50	90	25	100	15	100
6 ml F, 6 ml IP..	10	40	10	50	5	50	0	50	0	95
Stove oil no. 2:										
12 ml F, 0 ml IP..	60	95	70	95	75	95	85	100	100	100
6 ml F, 6 ml IP..	10	90	10	90	0	95	0	95	0	95
Stove oil no. 3:										
12 ml F, 0 ml IP..	50	90	40	95	40	95	40	100	30	100
6 ml F, 6 ml IP..	10	80	10	80	0	90	0	90	0	95
Stove oil no. 4:										
12 ml F, 0 ml IP..	60	80	50	90	40	90	50	100	75	100
6 ml F, 6 ml IP..	10	70	10	70	0	75	0	75	0	75

According to the experiments reported above, oils offer much promise as selective sprays in onions and flax, two crops not previously treated with such materials. Other selectivities may still exist unexplored by the experimental method. In one case observed, barley was killed in a culture containing wild mustard; this constitutes a reversal of the usual selectivity observed with dinitro sprays (Crafts, 1946).

Susceptibility of Weed Species to Selective Oils. As for selectivity with regard to weed species, mustard and fiddleneck are two of the most susceptible weeds; they are readily controlled with gasoline, stove oil, and Diesel oil. Pigweeds, goosefoot, and lambs' quarters are somewhat more difficult to kill; cow thistle and wild lettuce are rather resistant to oil sprays. Dense matted stands of chickweed are very stubborn. Grasses, including the cultivated and

wild cereals, are some of the most easily controlled weeds where oil sprays are used. In fact, the oils seem almost specific for grasses; and where fractions as heavy as stove oil and kerosene are used, the slowly developing chronic toxicity will often kill grass species after several weeks where, for the first few days, they seemed almost unharmed. Because kerosene seems particularly capable of causing such chronic injury without harming onions, flax, mustard, and other plants, it should receive more detailed study as a selective herbicide.

PHYSICAL PROPERTIES OF OIL AND APPLICATION METHODS

Properties of Water and Oils. The two common types of liquids used as herbicidal sprays, namely aqueous solutions and oils, have contrasting physical properties. Water is a highly polar compound. Its boiling and melting points are much higher than would be expected from its composition; its viscosity and surface tension are also extremely high.

The oils are much more usual in their physical properties. Their melting and boiling points and their viscosities are more commensurate with their molecular weights and composition; their surface tensions are only one third to one half that of water (table 54).

Plant surfaces are commonly cutinized or suberized. Cutin and suberin are fatty substances that resemble oils in their chemical affinities. For this reason aqueous sprays are repelled by most plant surfaces; they round up into spherical droplets and tend to run off. Even when atomized by high pressure and applied with great force, they do not readily stick to the waxy surfaces of many plants. If they do stick they stay in place or collect into droplets, presenting a minimum surface of contact.

Oils of the type used for weed spraying, being low in viscosity and surface tension and having affinities for fatty surfaces, wet plants rapidly and completely, tending to spread and to creep. They form thin films and move along plant surfaces, penetrating every crack and crevice. They gravitate downward so long as film thickness permits free movement; crowns of plants may be covered by oil that has been applied to foliage several inches above. Having covered the plant, an oil penetrates the cuticle and cell walls and comes into intimate contact with the cell protoplasm.

Properties of Plant Surfaces. Plants vary in structure, and certain reactions to aqueous and oil sprays reflect these differences. Most dicotyledonous plants have an open branching form with extended leaves and exposed growing regions. These plants, such as mustard and fiddleneck, are readily killed by aqueous sprays, even though their waxy surfaces are not easily wet. Grasses, on the other hand, have minutely ridged surfaces with waxy or siliceous coatings that repel water; their leaves are often vertical in position; and their growing regions are enclosed by the bases of older leaves. To penetrate such plants, oils have especially favorable properties. This may be one reason why oils tend to act as specific toxicants against grasses.

For reasons cited above, oils have proved invaluable for spraying grasses and mixed weeds along highways, railroads, and such places, where all weed growth is undesirable. Before World War II, the California highway system required the use of nearly a million gallons of Diesel oil per year for firebreak maintenance.

Creeping of Oils. For a more accurate study of the part played by the creeping of oils, an experiment was set up to test oil toxicity on tissues not actually sprayed. Four cultures having barley plants about 10 inches in height were selected. The first was sprayed in an upright position in the usual manner, with 6 ml of Diesel oil. Two others were laid on their sides; strings were tied around the plants at a level of 5 inches above the soil; and, with the lower halves shielded, the upper portions were sprayed until thoroughly saturated

TABLE 54
SOME PHYSICAL PROPERTIES OF WATER, OILS, AND SOME
COMMON ORGANIC LIQUIDS

Compound	Boiling point, ° Centigrade	Surface tension, dynes per cm at 20° C
Petroleum fractions.		
Naphtha	Up to 150	19-23
Kerosenes	150-300	23-32
Gas oils	250-325	28-29
Petroleum distillates*	Up to 100	19
Petroleum distillates*	100-150	23
Petroleum distillates*	150-200	25
Petroleum distillates*	200-250	26-5
Petroleum distillates*	250-300	28
Pure compounds		
Water	100.0	72.75 ± 0.5
Ethyl alcohol	78.5	22.27
Benzene	79.6	28.88
Cyclohexane	81.4	25.3
n-Hexane	69.0	18.43
Toluene	110.5	28.43
Ethyl benzene	136.5	29.20
o-Xylene	144.0	30.10
m-Xylene	139.0	28.90
p-Xylene	137.7	28.37
n-Octane	124.6	21.80

* Estimated from fig. 3, p. 149, International Critical Tables.

with oil. One of these was then placed in an upright position; the other remained on its side. The fourth was left unsprayed, as a control.

As injury developed on these cultures, it became apparent that the creeping of the Diesel oil down the stems has an important bearing upon the results of spraying. Whereas the oil on the plants that remained in a horizontal position moved a maximum of 2 inches toward the soil, the plants that were sprayed on the top half and then placed upright died completely, and the odor of Diesel oil could be detected in the tissues at the crown. When the experiment was repeated and one culture completely inverted, there was even less creeping toward the crown than in the horizontal position. Observing the oil on the plant set upright, one could see the thin film spreading down the leaves and into the narrow crevice between the leaf base and the inner leaf which it surrounds. Within 24 hours after spraying, if the youngest leaf at the tip of the barley plant was pulled out, it was found to be dead at the region of the secondary meristem.

This experiment leads to several deductions regarding Diesel oil as a general contact herbicide:

1. After being applied as a spray, such an oil will creep at least 5 inches down the grass stems into the crown; the weeds will die even after a poor job of spraying; whereas emulsions or aqueous sprays, because they lack the ability to creep, would only injure the regions actually hit.

TABLE 55

EXPERIMENTS ON THE EFFECTS OF CONTACT AND CREEPING OF OILS ON TOXICITY TO BARLEY; JANUARY 8, 1945

Material and method of application	Injury to barley, in per cent						
	1 day	2 days	4 days	5 days	7 days	11 days	13 days
Gasoline:							
Drops in top leaf	10	20	25	50	70	100	100
Drops on crown	0	5	5	10	50	95	100
Paint on crown	5	5	5	20	50	90	100
Horizontal spray + vertical position	75	90	80	90	90	90	90
Horizontal spray + horizontal position	50	75	60	90	90	90	90
Isoparaffin:							
Drops in top leaf	0	0	5	10	20	40	60
Drops on crown	0	0	0	0	10	50	90
Paint on crown	0	0	0	0	10	90	95
Horizontal spray + vertical position	0	0	10	50	90	100	100
Horizontal spray + horizontal position	0	0	10	25	50	50	90
Kerosene:							
Drops in top leaf	0	10	20	40	40	40	50
Drops on crown	0	0	0	10	20	90	100
Paint on crown	0	0	0	20	40	90	100
Horizontal spray + vertical position	0	5	25	75	95	100	100
Horizontal spray + horizontal position	0	5	15	40	40	50	90
Diesel oil:							
Drops in top leaf	0	5	15	25	30	40	40
Drops on crown	0	0	0	10	10	30	30
Paint on crown	0	0	0	10	10	10	10
Horizontal spray + vertical position	0	0	25	50	75	95	100
Horizontal spray + horizontal position	0	0	25	50	60	60	90

2. The oil is highly toxic: even the minute quantity present in the thin film will kill the meristematic region.

3. Toxicity is largely chronic; acute toxicants, when present in such small quantities, are not potent enough to kill the tissues or they evaporate before killing is complete. These results largely explain the wide satisfaction given by Diesel oil as a general contact spray.

Role of Application Method. One further experiment was performed to compare the abilities of different oils to kill barley plants when various methods of application are used. In table 55, which presents the results, several points of interest may be noted. When the oil was applied by depositing several drops in the funnel formed by the top leaf, gasoline proved the most toxic. This finding reflects the intense acute activity of this oil. Penetrating rapidly, the gasoline evidently killed the meristematic region within a short time. Applied on the outside of the crown, or sprayed on, it evaporated before it had time to react with all the tissues.

Isoparaffin was most effective when applied as a spray and allowed to run down into the crown. Killing was by slow chronic action. Kerosene was somewhat more toxic and was most effective when sprayed on and allowed to run into the crowns. Diesel oil painted on the outside of the crowns was very ineffective. It apparently penetrates living plant tissue very slowly. Applied as a spray and allowed to run down all the leaves, it eventually permeated the whole crown and killed the tissues completely.

Judging from these considerations, one must, in preparing an effective herbicidal oil, balance the factors of toxicity, viscosity, surface and interfacial tension, and volatility in order to accomplish a given task.

Suitability of Commercial Fractions to Weed Control. The natural fraction used for Diesel fuel happens to fit many of the requirements for a general contact herbicide. If, in order to improve its preignition properties and lessen smoking, one reduces the aromatic and olefinic content, this refining will lower its value as a herbicide. Another fraction—stove oil—has proved valuable as a selective spray in vegetable crops. If its A.P.I. gravity rating drops only a few degrees, or if aromatics are extracted to reduce smokiness in burning, this fraction will no longer fit requirements for killing weeds.

In most oils used for weed control, the components responsible for acute toxicity constitute a relatively small portion of the total volume; the remaining components—aliphatic hydrocarbons—are comparatively low in toxicity and act principally as carriers. In future work on the compounding of herbicidal oils, a higher degree of control may well be exercised to produce special mixtures adapted to weeds and crops. Conceivably, workers may balance the toxicity resulting from olefinic and aromatic content against the volume as related to convenience in application. In addition, they may regulate the spreading and penetrating properties by control of viscosity and surface tension. By such methods, perhaps, they will be able to produce new and better herbicides for both selective and general weed control.

PROPERTIES OF EMULSIONS

Since the unsaturated compounds in an oil fraction are largely responsible for the toxicity, apparently the bulk of most oils is relatively inert as regards injury to the plant and therefore acts principally as a carrier. If, therefore, an oil fraction carries more toxicity than is required to destroy the weed growth that it will conveniently cover, it might be extended, and its toxicity more efficiently utilized, by emulsification with water.

In the early trials with Diesel oil for roadside weed control, many attempts were made to improve the spread and cover by adding water and emulsifiers so that more volume would be available at little additional cost. Practically all such attempts failed. In view of the experiments just described, the reason seems clear. Two faults may be found with the use of Diesel-oil emulsions. First, the oil has little excess toxicity; when efficiently sprayed upon weeds it carries just about enough toxicity to kill the vegetation it will cover. Second, no emulsion of oil and water has the affinity for plant surfaces that straight oil has; if more than a covering film is applied, the emulsion collects in droplets and runs off, and any oil included in such droplets is lost.

Uses of Emulsions. At the outset of the experimental program on oils, it seemed that there might be several possible uses for emulsions. First, if the selective toxicant responsible for the differential killing of weeds in carrot crops could be separated, it might be applied as an emulsion in water, and thus the cost of the oil carrier would be saved. Second, a highly toxic oil such as Avon Weed Killer might be extended to make more efficient use of its toxicity. Third, with the discovery of the high toxicity of such organic compounds as dinitro-*o*-cresol, dinitro-*sec*-butylphenol, dinitro-*sec*-amylphenol, and pentachlorophenol, all of which are oil-soluble, it seemed possible that emulsions carrying these toxicants in solution in the oil phase might prove effective contact herbicides. Experiments to test these propositions have been conducted.

TABLE 56

TOXICITY OF STOVE-OIL EMULSIONS* OF VARYING COMPOSITION TO CARROTS, BROAD-LEAVED WEEDS, AND GRASSES; JANUARY 30, 1943

Amounts of stove oil and water in the emulsion	Injury to carrots (C), broad-leaved weeds (BW), and grasses (G), in per cent								
	1 day			3 days			6 days		
	C	BW	G	C	BW	G	C	BW	G
1 ml SO, 5 ml W.....	0	20	20	0	20	20	0	20	20
2 ml SO, 4 ml W.....	0	60	50	0	60	50	0	75	50
3 ml SO, 3 ml W.....	0	90	90	0	90	90	0	95	90
4 ml SO, 2 ml W.....	10	100	100	10	100	100	5	95	95
5 ml SO, 1 ml W.....	50	100	100	40	100	100	40	95	100
6 ml SO, 0 ml W.....	0	100	75	0	100	100	0	95	100

* Emulsions were stabilized with sodium lauryl sulfate.

Tests of Emulsions. The first test involved some stove-oil emulsions stabilized with sodium lauryl sulfate and sprayed on carrots. Table 56 presents the results. Sodium lauryl sulfate was present in the water at a concentration of $\frac{1}{10}$ per cent by weight.

If the data of this table are compared with those of table 3, it will be found that the emulsions were somewhat more toxic than equivalent volumes of straight oil. In either case, however, 4 to 5 ml of oil was required to kill the weeds, and emulsions containing these amounts of oil were more toxic to carrots than were the straight oil sprays.

The next experiment tested emulsions of Avon Weed Killer and Diesel oil. Both unstabilized and stabilized emulsions of the weed killer were used. The results (table 57) show that this highly aromatic fraction (Avon Weed Killer) is selective if diluted with odorless kerosene. It is also selective when used as an emulsion in water. The emulsion stabilized by adding $\frac{1}{10}$ per cent of Vatsol (OT 70 per cent clear) by weight to the water was somewhat more toxic to both weeds and carrots than was the unstabilized mixture. Although the 50:50 Diesel oil and water mixture was selective, injury developed slowly and was not nearly complete on broad-leaved weeds.

Emulsions on Flax. Carrying the same idea further, an extensive experiment was conducted with gasoline, stove-oil, Diesel oil, and Avon Weed Killer

used in dilutions with odorless kerosene and as emulsions both stabilized and unstabilized. The test plants were flax, mustard, and barley. The cultures were young, and the plants succulent and rapidly growing. Selectivity was poor throughout, and no mixture looked good enough to offer commercial possibilities. The only generalization derived from the experiment was that the use of oils, as emulsions, on young tender flax is a hazardous procedure.

TABLE 57

TOXICITY OF AVON WEED KILLER AND DIESEL OIL AT VARIOUS CONCENTRATIONS TO CARROTS, BROAD-LEAVED WEEDS, AND GRASS; FEBRUARY 22, 1944

Oil fraction and amounts of fraction and diluent applied per culture	Injury to carrots (C), broad-leaved weeds (BW), and grass (G), in per cent											
	1 day			3 days			8 days			16 days		
	C	BW	G	C	BW	G	C	BW	G	C	BW	G
Avon Weed Killer and odorless kerosene:												
5 ml F, 5 ml OK.....	25	100	95	40	100	100	40	100	100	20	100	100
2½ ml F, 7½ ml OK.....	0	100	90	5	100	100	5	100	100	5	100	100
1½ ml F, 8½ ml OK.....	0	75	60	0	70	75	0	80	100	0	75	100
¾ ml F, 9¾ ml OK.....	0	20	5	0	20	10	0	25	20	0	20	50
Avon Weed Killer and water emulsion:												
2½ ml F, 7½ ml W.....	0	75	75	10	95	90	10	95	75	5	75	75
1½ ml F, 8½ ml W.....	0	60	50	0	90	60	0	75	60	0	60	60
¾ ml F, 9¾ ml W.....	0	25	30	0	40	50	0	50	50	0	30	90
Avon Weed Killer and Vatsol solution:*												
2½ ml F, 7½ ml V.....	20	90	90	25	100	90	25	100	90	15	100	95
1½ ml F, 8½ ml V.....	0	75	90	5	95	90	5	100	75	5	100	80
¾ ml F, 9¾ ml V.....	0	60	60	0	50	75	0	50	70	0	50	75
Diesel oil and water emulsion:												
5 ml F, 5 ml W.....	0	30	50	5	40	75	5	50	90	5	50	100
2½ ml F, 7½ ml W.....	0	15	20	0	20	50	0	25	50	0	25	70
1½ ml F, 8½ ml W.....	0	10	5	0	10	10	0	10	25	0	10	90

* Vatsol solution: Vatsol OT 70 per cent clear was used at a concentration of 1/10 per cent by volume.

Emulsions on Onions. Since a selective kill of grass and weeds in onions seemed possible (see tables 50, 51, 52, and 53), a series of dilutions and emulsions of xylene, TS-28 solvent, Edeleanu extract, and gasoline was tested on onions, grass, and broad-leaved weeds. Table 58 presents the results.

The emulsions used in this experiment were highly toxic to grasses, somewhat less toxic to broad-leaved weeds, and less toxic to onions. Xylene and white gasoline were selective enough to look promising; the highly aromatic TS-28 and the Edeleanu extract were too toxic, even at the lowest concentrations used, to be commercially feasible. It was apparent during this experiment that all the emulsions killed faster than did the straight oil mixtures. Since, however, they did not penetrate so well, grasses tended to recover toward the end of the experiment. The emulsions never showed higher selectivity than the oil mixtures.

In the use of emulsions the phase relations are important. When the water makes up the external phase, the emulsion presumably will react on the plant like an aqueous spray; when oil constitutes the external phase, the spray

should spread like an oil. The producers of Avon Weed Killer submitted two samples for testing—one of the usual type and one containing reagents that caused it to form the water-in-oil or invert type of emulsion. Emulsions of

TABLE 58
TOXICITY OF AROMATIC FRACTIONS TO ONIONS, GRASS, AND BROAD-LEAVED WEEDS;
APRIL 14, 1944

Oil fraction and amounts of fraction and diluent applied per culture	Injury to onions (O), grass (G), and broad-leaved weeds (BW), in per cent														
	1 day			3 days			5 days			10 days			15 days		
	O	G	BW	O	G	BW	O	G	BW	O	G	BW	O	G	BW
Xylene and odorless kerosene:															
5 ml F, 5 ml OK.....	10	75	50	10	90	50	10	95	50	5	100	50	5	100	50
4 ml F, 6 ml OK.....	10	75	30	10	90	50	10	95	50	5	100	75	5	100	100
3 ml F, 7 ml OK.....	0	40	25	0	60	20	0	75	20	0	85	10	0	95	25
Xylene and Vatsol solution*:															
5 ml F, 5 ml V.....	30	90	80	30	90	80	20	95	80	5	100	80	5	100	80
4 ml F, 6 ml V.....	20	80	70	20	80	70	10	50	70	5	50	70	0	50	70
3 ml F, 7 ml V.....	15	50	30	15	70	30	20	50	50	5	50	50	5	75	70
TS-28 solvent and odorless kerosene:															
5 ml F, 5 ml OK.....	50	95	90	50	95	95	60	100	95	40	100	100	40	100	100
4 ml F, 6 ml OK.....	30	50	20	30	90	50	30	100	50	15	100	50	20	100	50
3 ml F, 7 ml OK.....	25	50	40	15	70	40	10	90	40	5	100	40	20	100	50
TS-28 solvent and Vatsol solution*:															
5 ml F, 5 ml V.....	60	90	90	50	95	90	40	80	95	20	70	100	30	80	100
4 ml F, 6 ml V.....	40	90	95	40	90	95	30	70	90	15	80	95	30	80	100
3 ml F, 7 ml V.....	40	80	90	30	75	90	30	80	90	15	80	100	20	80	100
Edeleanu extract (E-1295) and odorless kerosene:															
5 ml F, 5 ml OK.....	50	90	80	50	90	80	60	100	100	75	100	100	75	100	100
4 ml F, 6 ml OK.....	50	90	95	50	95	95	50	100	100	60	100	100	60	100	100
3 ml F, 7 ml OK.....	40	95	90	40	95	95	75	100	100	60	100	100	100	100	100
Edeleanu extract (E-1295) and Vatsol solution*:															
5 ml F, 5 ml V.....	60	95	95	75	95	95	75	90	100	75	100	100	75	100	100
4 ml F, 6 ml V.....	60	95	95	75	95	95	60	90	100	80	80	100	60	90	100
3 ml F, 7 ml V.....	75	95	95	90	95	95	95	100	100	90	100	100	90	100	100
White gasoline and odorless kerosene:															
10 ml F, 0 ml OK.....	50	95	75	40	90	60	30	90	40	15	90	30	20	90	40
7½ ml F, 2½ ml OK.....	5	95	75	5	95	75	5	100	50	0	100	50	0	100	80
5 ml F, 5 ml OK.....	0	0	20	0	0	15	0	50	20	0	75	20	0	50	20
White gasoline and Vatsol solution*:															
7½ ml F, 2½ ml V.....	50	90	95	50	95	95	40	50	95	40	80	100	30	70	100
5 ml F, 5 ml V.....	20	75	90	40	70	95	30	30	95	20	30	100	10	30	100

* Vatsol OT 70 per cent clear was used at a concentration of 1/10 per cent by volume.

both types were prepared at varying proportions of oil to water and applied to plants. Total volume was the same in all cases and did not provide excess solution for runoff.

Since, under these conditions, toxicities of the two types proved identical, apparently one can gain no advantage by making an invert emulsion where spray volume is controlled so that no runoff occurs. Emulsion of the invert type, however, did spread somewhat more rapidly than the oil-in-water type.

Use of Sulfur in Diesel-Oil Emulsions. During the experiments described above, a report was received from Ethelbert Johnson, District Supervisor of

TABLE 59

TOXICITY OF DIESEL OIL AND DIESEL-OIL EMULSIONS WITH AND WITHOUT SULFUR
TO BARLEY AND MUSTARD; JANUARY 30, 1945

Mixture, culture, and amount applied per culture	Injury to barley (B) and mustard (M), in per cent							
	1 day		3 days		6 days		10 days	
	B	M	B	M	B	M	B	M
Diesel oil and water, 50:50:								
Young barley and mustard:								
3 ml.....	0	5	0	10	10	20	10	20
4 ml.....	0	5	5	50	25	75	75	80
6 ml.....	0	20	25	75	60	95	100	95
9 ml.....	10	40	50	85	90	100	100	95
12 ml.....	20	50	75	90	95	100	100	100
Tall barley:								
6 ml.....	0	..	5	..	20	..	50	..
9 ml.....	0	..	10	..	40	..	75	..
12 ml.....	0	..	20	..	70	..	90	..
Tall mustard:								
9 ml.....	..	15	..	25	..	30	..	40
18 ml.....	..	25	..	75	..	75	..	75
Diesel oil and water, 50:50 plus sulfur:								
Young barley and mustard:								
3 ml.....	0	20	25	75	75	90	95	100
4 ml.....	5	25	75	75	90	90	100	90
6 ml.....	10	75	90	85	95	100	100	100
9 ml.....	40	90	95	100	100	100	100	100
12 ml.....	60	90	100	100	100	100	100	100
Tall barley:								
6 ml.....	0	..	25	..	40	..	50	..
9 ml.....	5	..	40	..	75	..	95	..
12 ml.....	10	..	60	..	95	..	100	..
Tall mustard:								
9 ml.....	..	40	..	80	..	80	..	80
18 ml.....	..	65	..	95	..	95	..	95
Diesel oil straight:								
Young barley and mustard:								
1½ ml.....	5	10	25	50	60	75	90	75
2 ml.....	5	20	50	50	75	75	90	75
3 ml.....	15	75	75	100	95	100	100	100
4½ ml.....	20	75	90	100	100	100	100	100
6 ml.....	60	90	95	100	100	100	100	100
Tall barley:								
3 ml.....	0	..	20	..	60	..	60	..
4½ ml.....	5	..	40	..	75	..	95	..
6 ml.....	10	..	60	..	75	..	80	..
Tall mustard:								
4½ ml.....	..	25	..	75	..	80	..	80
9 ml.....	..	40	..	95	..	95	..	95

Weed and Rodent Control, State of California: spray operators in his territory were using 50:50 emulsions of Diesel oil and water containing 0.6 per cent sulfur, with results comparable with those secured with pure Diesel oil. An experiment to test this question was designed; the results appear in table 59. Sulfur was included in the second emulsion at a rate of 2 grams in 332 ml of the 50:50 mixture. No stabilizer was used.

From table 59 it is apparent that the emulsion plus sulfur was more toxic than the one without; in fact, it was equal to the straight Diesel oil, which indicates that the 2 grams of sulfur added as much killing power to the mixture as 166 ml of oil would have done.

In further correspondence Mr. Johnson mentioned that the addition of 2 or 3 gallons of high-asphalt fuel oil per 100 gallons will change a 50:50 emulsion of Diesel oil and water from an oil-in-water to a water-in-oil type—in other words, will bring about inversion. Adding 5 pounds of dusting sulfur to 100 gallons of the same mixture had a similar effect. Mr. Johnson concluded that inversion caused the increase in toxicity; hence the effect was physical rather than chemical. In the experiment described, however, there was a decided difference between the action of the mixture containing sulfur and the others. This mixture proved more toxic to the young cultures than an equivalent volume of oil. The series with sulfur resembled that with straight oil for about 6 days; then the plants sprayed with the sulfur mixture started to turn light in color; and by the end of the observation period they were almost completely bleached. Evidently the sulfur had oxidized and produced the characteristic chemical action of SO_2 . This result justifies further study, since it indicates a method for materially lowering the cost of using Diesel oil as a contact herbicide.

FORTIFIED OILS AND OIL EMULSIONS

As explained in the foregoing sections, the naturally occurring toxicants in oil fractions are not highly toxic. To kill weeds, the concentration of acute toxicants must be in the order of 20 per cent or more; chronic toxicants, though more effective, act slowly. In spite of the expense involved, the use of acute toxicants in the boiling range of third-structure gasoline, kerosene, and stove oil as selective weed killers in vegetable crops seems justified. Unfortunately, however, these toxicants are too costly to serve as general contact herbicides.

Slow Action of Heavy Oils. The heavier oils are much less spectacular in their effects. Even though they kill, the action takes many days; and in some situations this is a real disadvantage—for instance in preparing firebreaks on highways, where vegetation must be killed rapidly in preparation for burning, before the surrounding plants become dry enough to carry fire. The refining of Diesel and similar fuel oils tends to lower acute toxicity and to make their killing action even slower.

Oil-tolerant Weed Species. A further drawback to the use of oils as general contact herbicides is the fact that weeds of the family Umbelliferae are oil-tolerant and many other species are not readily killed. Fennel and star thistle have invaded the firestrips along California highways; in some places they have become as serious a hazard as the original vegetation.

Fortification of Oils. The nitro- and chlorophenols are highly effective acute toxicants. Dinitro-*o*-cresol, dinitro-*sec*-butylphenol, dinitro-*sec*-amylphenol, and pentachlorophenol are all valuable fortifying agents for increasing the speed and thoroughness of killing by oil sprays. Furthermore, if one makes a concentrated solution of one of these toxicants in oil, the killing potential is so high that it greatly exceeds the covering ability of the liquid; hence the solution can be effectively extended by emulsification.

In general, polar compounds are more soluble in polar solvents, nonpolar compounds in nonpolar solvents; and the more the solute resembles the solvents in chemical properties, the greater the solubility. Hence the solution of the substituted phenols in oils depends upon two factors: (1) the content of polar compounds (mainly olefinic and aromatic) in the oil; (2) the type and degree of substitution in the phenyl ring of the phenol compound.

It was found that dinitrocresol dissolves to the extent of about $\frac{1}{2}$ per cent in kerosene, a highly aliphatic solvent; dissolves to about 1 per cent in stove oil containing around 20 per cent unsaturates; is over 10 per cent soluble in xylene, an aromatic hydrocarbon. Pentachlorophenol is 8 per cent soluble in Diesel oil; about 16 per cent in Avon Weed Killer.

TABLE 60

TOXICITY OF EMULSIONS OF STOVE OIL IN WATER CONTAINING SODIUM PENTACHLOROPHENATE TO BARLEY AND ANNUAL BLUEGRASS; JANUARY 24, 1944

Amount of stove oil (SO) applied per culture with 10 ml stock solution,* and total volume applied	Total injury to barley (B) and annual bluegrass (BG), in per cent									
	1 day		2 days		3 days		11 days		14 days	
	B	BG	B	BG	B	BG	B	BG	B	BG
0.025 ml SO, total 10.025 ml	0	5	0	40	0	60	0	90	0	100
0.050 ml SO, total 10.05 ml.	0	10	5	35	5	60	5	75	5	100
0.1 ml SO, total 10.1 ml	5	20	10	60	10	75	10	100	10	100
0.2 ml SO, total 10.2 ml.	10	25	20	75	15	85	10	100	10	100
0.3 ml SO, total 10.3 ml	15	30	25	70	25	85	10	100	10	100
0.4 ml SO, total 10.4 ml.	15	30	25	70	25	90	10	100	10	100
0.5 ml SO, total 10.5 ml.	20	50	40	80	40	90	15	100	15	100
0.6 ml SO, total 10.6 ml.	30	60	60	90	50	95	30	100	25	100
0.8 ml SO, total 10.8 ml.	50	70	60	90	60	95	40	100	40	100
1.0 ml SO, total 11.0 ml.	75	80	75	90	50	95	40	100	40	100

* This stock solution contained 1 per cent sodium pentachlorophenate, $\frac{1}{4}$ per cent aluminum sulfate, and $\frac{1}{10}$ per cent Vatsol (OT 70 per cent clear) by weight.

Considering the solubility of substituted phenols in oils, the longer the chain substitution of a dinitro-substituted phenol, the greater the solubility of the compound in oil. Dinitro phenol is only slightly soluble in Diesel oil, dinitrocresol is soluble to about 1 per cent, and dinitro-*sec*-butylphenol to around 20 per cent; the amyl compound is an oily liquid miscible in all proportions at room temperature. If suitable emulsifiers are added, the dinitro-*sec*-amylphenol will make a ready-mix type of emulsion of high stability in water without the addition of oil.

Tests of Fortified-Oil Emulsions. Many experiments were performed exploring the possible combinations of toxicants, oils, and stabilizers. Table 60 presents data from one such test. In this a stock solution containing 1 per cent sodium pentachlorophenate, $\frac{1}{4}$ per cent aluminum sulfate, and $\frac{1}{10}$ per cent Vatsol (OT 70 per cent clear) by weight in water was made up. To this were added varying volumes of oil, as indicated in the table. Oil volume is in percentage by volume. The data show a consistent increase in toxicity with increasing volume of oil through the whole series. Results in the field confirm this relation.

There was an extended series of tests on the relation between concentration of the activated sodium pentachlorophenate and toxicity at three levels of stove-oil concentration—namely 2, 4, and 8 per cent. The results showed that rather large increases in concentration of either of these essential constituents are required to bring about complete killing of grasses, as compared with results in the 80 to 95 per cent toxicity region. With 2 per cent oil an increase of pentachlorophenate concentration from $\frac{1}{2}$ per cent up to 1 per cent significantly heightened the toxicity; further increase in concentration was useless. At 4 per cent oil, increase of phenate toxicity from $\frac{1}{2}$ per cent to $\frac{3}{4}$ per cent was effective; further increase was useless. At 8 per cent oil, toxicity was con-

TABLE 61

TOXICITY OF STOVE OIL AND EMULSIONS OF STOVE OIL AND WATER CONTAINING ACTIVATED SODIUM PENTACHLOROPHENATE AND VATSOL TO BARLEY; FEBRUARY 2, 1944

Spray and amount applied per culture	Injury to barley, in per cent					
	1 day	2 days	3 days	5 days	9 days	13 days
Stove oil emulsion:*						
5 ml.....	60	60	60	60	70	75
10 ml.....	75	80	80	80	85	90
15 ml.....	90	95	95	95	98	98
20 ml.....	90	95	95	95	98	99
25 ml.....	95	97	99	99	100	100
Stove oil straight:						
5 ml.....	50	60	60	70	70	75
10 ml.....	60	90	90	90	90	95
15 ml.....	70	90	95	100	100	100
20 ml.....	75	95	100	100	100	100
25 ml.....	90	100	100	100	100	100

*Stove-oil emulsion, percentages in water:

Sodium pentachlorophenate.....	1.0 per cent by weight
Aluminum sulfate.....	0.25 per cent by weight
Vatsol (OT 70 per cent clear).....	0.10 per cent by weight
Stove oil.....	2.0 per cent by weight

stant throughout the range $\frac{1}{2}$ to 2 per cent of phenate; and it was as high as the toxicity at higher phenate levels with less oil.

Table 61 compares this type of emulsion with straight stove oil. At the higher volume rates the emulsion compared very favorably with straight stove oil. If the oil content had been raised to 6 per cent by volume, the liquids would have been almost identical in toxicity.

In subsequent experiments, pentachlorophenol dissolved directly in the oil of an emulsion proved to be as effective as the activated sodium salt dissolved in the aqueous phase. When Avon Weed Killer was used, a solution containing $\frac{1}{2}$ per cent pentachlorophenol in 3 per cent oil with 0.1 per cent Vatsol proved highly toxic against star thistle and grass at 15 ml per culture; in fact, this emulsion was equivalent, volume for volume, with stove oil and Diesel oil as a contact killer.

When dinitro-*sec*-butylphenol became available for testing, a series of experiments indicated it to be roughly four times as toxic as dinitrocresol and pentachlorophenol, somewhat more toxic than dinitro-*sec*-amylphenol. Being readily soluble in oil, it offered much promise as a contact herbicide.

Quantitative Relations of Oil and Fortifying Agent. One problem in concocting a general contact herbicide from oil and a dinitro compound is to arrive at the most economical mixture of the two from the herbicidal standpoint. Tests were conducted using a mixture of oil and stabilizer with and without the dinitro compound. When these were mixed in varying propor-

TABLE 62
TOXICITY OF EMULSIONS CONTAINING DINITRO-*sec*-BUTYLPHENOL AND
DIESEL OIL, OR DOW CONTACT HERBICIDE AND DIESEL OIL,
TO GRASSES; DECEMBER 5, 1944

Concentrations, in per cent*	Injury to grasses, in per cent					
	1 day	2 days	3 days	4 days	6 days	8 days
Dinitro- <i>sec</i> -butylphenol and Diesel oil emulsions:						
0.68 per cent DN:						
19.3 per cent DO	75	98	100	100	100	100
9.3 per cent DO	75	98	98	98	98	98
4.3 per cent DO	60	98	98	98	98	98
0.34 per cent DN:						
9.6 per cent DO	60	95	100	100	100	100
4.6 per cent DO	60	95	95	95	95	95
2.2 per cent DO	50	95	98	98	98	98
0.17 per cent DN:						
4.8 per cent DO	40	90	90	90	90	90
2.3 per cent DO	40	90	95	95	90	90
1.1 per cent DO	40	90	95	95	95	95
0.08 per cent DN:						
2.4 per cent DO	20	80	90	90	90	90
1.2 per cent DO	20	75	90	90	90	90
0.5 per cent DO	25	80	95	95	95	90
Dow Contact Herbicide and Diesel oil emulsions:						
0.68 per cent DN:						
19.3 per cent DO	75	98	98	98	98	98
9.3 per cent DO	75	98	98	98	98	95
0.34 per cent DN:						
9.6 per cent DO	60	95	95	95	95	90
4.6 per cent DO	60	95	95	98	95	95
0.17 per cent DN:						
4.8 per cent DO	40	90	95	95	95	95
2.3 per cent DO	40	90	95	95	98	98
0.08 per cent DN:						
2.4 per cent DO	20	75	90	90	90	90
1.2 per cent DO	20	75	90	90	90	90

* Volume applied per culture, 10 ml.

tions, several combinations were obtained. These were designed to compare in concentration with standard dilutions of Dow Contact Herbicide, a proprietary mixture containing dinitro-*sec*-butylphenol in oil with added stabilizer. Table 62 presents the results.

As these data show, a fourfold reduction of oil concentration from 19.3 per cent to 4.3 per cent has less effect than a fourfold reduction in concentration of the dinitro compound. On the other hand, a twofold reduction in dinitro concentration changed the toxicity very little; further reduction had an appreciable effect. The evident conclusion is that the dinitro concentration of the solution as used in the field should be not less than $\frac{1}{3}$ per cent; the oil

concentration may vary through rather wide limits without seriously affecting results. These conclusions agree with field experience. On broad-leaved weeds the contact herbicide, at $\frac{1}{3}$ per cent dinitro and around 3 per cent oil, is effective; on grasses, as in table 62, the kill is not complete. Experience has shown that increasing the dinitro concentration has little benefit as far as grasses are concerned; increasing the oil content to around 10 per cent provides the necessary qualities to make the injury complete. Judging from the experiments cited in this publication, the extent of kill on grasses is a matter of spreading and penetration of the spray solution rather than one of absolute toxicity.

The fortifying of oils with substituted phenol compounds has a single purpose—namely, to increase toxicity so that a smaller volume will be required per unit area. If the phenolic toxicant is less expensive per unit of toxicity, two savings are made: less oil is used; less volume need be hauled, transferred, and pumped through the spray rig.

Volume Relations of Emulsions. Where the vegetative growth is rank, there is a definite limit to the reduction in volume that may be utilized; if a relatively large volume is required, the oil carrier may be used in greater amount, or the highly fortified oil may be extended by emulsification. If grasses make up a large portion of the vegetation being treated, the oil concentration in the emulsion should be kept above 6 per cent by volume; 10 per cent is better. Where the growth is largely broad-leaved weeds, the oil content may be reduced to as low as 2 per cent of the emulsion. Or the oil solution may be applied directly in low volume. For example, a fortified oil has been applied by airplane at a rate of 15 gallons per acre to control young weed growth in a field where onion bulbs had been planted for seed production. Protracted rains had rendered the field too muddy to spray by ground rig. The onion tops were slightly injured, but recovered completely. Weeds were set back until the field was dry enough for cultivation.

Fortified Diesel oil has proved valuable as a selective spray for controlling annual weeds in alfalfa fields. Tried first in 1945, this method was used on several thousand acres of alfalfa during the 1946 season. It has a twofold benefit: it eliminates weeds in the first cutting, rendering this cutting one of the most valuable; tillage operations that tend to spread alfalfa wilt may be avoided. Spraying was done both by ground rigs and by airplane, and applications were made (in central California) in January and February while the alfalfa was dormant.

Oil Carriers for 2,4-D. Oils offer promise as carriers for the new 2,4-D weed killers. An experiment using the methyl ester of 2,4-D in Diesel oil, stove oil, kerosene, and *n*-cetane on nut grass gives definite indication that the nontoxic carriers are most effective. The oil toxicity of the first two apparently interfered with the action of the 2,4-D: the nut-grass plants resprouted promptly and vigorously, whereas those sprayed with the kerosene and cetane solutions, though they died slowly, did not resprout. Aqueous sprays of the salts of 2,4-D have proved less effective on nut grass than these oil solutions of the ester. Even though emulsified with small amounts of Diesel oil and stabilized with wetting agents that materially improve the spreading and penetrating powers, the aqueous solutions have had less lasting effects.

Several observers have reported that addition of small amounts of oil to aqueous solutions of 2,4-D increases toxicity, presumably owing to an improvement in spreading and penetration. This effect has been most pronounced on such weeds as cattail, tule, bur-reed, and similar species that are difficult to wet with straight aqueous sprays. Diesel and stove oils have been used for this purpose. The experiments on nut grass indicate that kerosene would be more effective because the acute toxicity of the less refined oils causes rapid injury and prevents translocation of the 2,4-D.

DISCUSSION OF OIL TOXICITY

Anyone familiar with toxicity testing will see that the foregoing text is a progress report. Although many of the questions that prompted the work have been answered, as many or more new problems have been posed. Most of the answers that have been given are empirical; they are practical answers to the grower's immediate problems. For instance, we now know that stove oil is suitable for selective weeding in carrots because it falls within the proper gravity range; Diesel oil is too heavy and causes chronic toxicity to which the crop plants are not tolerant; a fraction even lighter than stove oil would be highly desirable (more selective and less persistent); the principal drawbacks in the use of such a fraction are increased cost and the hazard of fire from its low flash point and high volatility.²²

Diesel oil is valuable as a general contact herbicide because of its low interfacial tension on plants and because its content of chronic toxicants is effective against grasses. Although highly aromatic residues are somewhat more active than Diesel oil, they have little practical advantage because of their limited covering power; their high toxicity cannot be completely utilized without the use of extenders; emulsification does not solve the problem where grasses are involved; dilution with oil is too expensive under a price ceiling set by Diesel fuel.

One may greatly increase the acute toxicity of any oil by adding fortifying agents. Such fortified oils may be used advantageously where large weedy grasses are not a major part of the weed population. They have proved effective in the weeding of ditchbanks; they kill all the weeds where wild carrot, fennel, and other oil-tolerant species are involved; they shorten the time required for killing weeds for mosquito control; they have solved the problem of controlling small annual weeds in dormant alfalfa and among onion bulbs planted for seed production. By means of airplanes, large alfalfa acreages can be covered in a short time and application can be made when moisture conditions prevent the operation of a ground rig.

The examples given above illustrate some of the information that has resulted from the research on oils. There still remain many problems concerning the fundamental responses of plants to oil application. Foremost among these is the nature of oil toxicity.

Nature of Oil Toxicity. As pointed out by de Ong, Knight, and Chamberlin (1927), oils may have two rather distinct types of toxicity: acute, caused by low-boiling oils; and chronic, caused by high-boiling fractions. Acute toxicity is usually apparent within 24 hours and is often fatal within 48. Chronic

²² See footnote 6, p. 84.

injury causes defoliation of citrus trees that begins within a few days and continues for weeks. It is evidenced in weed cultures as a slow yellowing of leaves, killing of the meristems of grasses, stunting, increased susceptibility to mildew infection, and finally death. Oils that are chronically toxic to grasses often cause a shift from negative to positive geotropism; whether this physiological response has any direct relation with chronic toxicity is yet to be determined.

De Ong, Knight, and Chamberlin (1927) concluded that the severe injury to citrus trees by the use of lubricating oils was associated with a high content of unsaturated hydrocarbons; sulfuric acid treatment of an oil removes aromatic, olefinic, resin, and sulfur compounds; such treatment has greatly reduced injury from the oils. As these writers have pointed out, among unrefined oils low-boiling ones are safer to use than heavier fractions because they evaporate quickly; but, for this same reason, they are not effective against scale insects.

Tucker (1936), from studies on the toxicity of fairly heavy oils (viscosity, Saybolt at 100° F, 77 to 420 sec.) on apricot leaves, concluded that the toxic agents were asphaltogenic acids, generated in the oils as a result of exposure to light and oxygen. He proposed that unsaturates are no more toxic than saturates but that formation of asphaltogenic acids is proportional to the amount of unsaturates present in a fraction.

Since Tucker worked only with oils that caused chronic toxicity, most of them highly refined, one can understand the reason for his conclusions. In contrast, many experiments reported in the present paper, including some with unrefined light fractions, many with pure compounds, and some with both unrefined and refined heavy fractions, prove that unsaturates are much more toxic than saturates.

Types of Oil Toxicity. An analysis of our results in the light of Tucker's conclusions will emphasize the fact that several types of toxicity are involved:

1. Acute toxicity caused by low-boiling unsaturates. This type is exhibited by gasoline and the light ends of stove oil; also by benzene, toluene, xylene, cyclohexene, cyclohexane, and many of their derivatives. It is shown in extreme form by Edeleanu extract and Avon Weed Killer. This is the toxicity to which members of the carrot family are relatively immune. Such toxicity is violent and nonpersistent because the compounds causing it are relatively volatile and soon leave the plant.

2. Chronic toxicity due to high-boiling unsaturates. This type results from application of Diesel oil and heavy fuel oils. It may also be caused by high-boiling aromatic hydrocarbons, such as the isopropyl benzenes. Its expression may vary through a wide range of time if the toxicants vary widely in concentration in different fractions. For instance, plants may be rapidly killed by heavy aromatic and olefinic fractions that are high in these unsaturates; they may require many days to respond to low concentrations when these are diluted with compounds of low toxicity. Though differences in tolerance occur among plants, even carrots succumb to chronic toxicants in sufficient concentration. Grasses are particularly susceptible to them. Symptoms may develop slowly; they consist of chlorosis, killing of meristems, cessation of growth, an unthrifty appearance, and high susceptibility to fungus attack.

3. Acute toxicity shown by highly refined fractions of low-boiling petroleum distillates that have been exposed to light for some time. This type of toxicity develops in gasoline and kerosene upon standing exposed to light. It is non-selective, violent, and nonpersistent because it is caused by compounds of high volatility. It develops, for example, in the isoparaffinic fractions reputed to be 100 per cent unsulfonated, and also in the unsulfonated residues of gasoline and kerosene. It was absent in stove oil, and stove-oil temperature cuts.

4. Chronic toxicity shown by highly refined fractions of high-boiling petroleum distillates that have been exposed to light. This toxicity developed in mineral seal oil, heavy isoparaffin, and—to a slight extent—in *n*-cetane. This is the type of toxicity studied by Tucker and attributed to asphaltogenic acids. It is undoubtedly caused by some products of oxidation of the oils; the exact nature of these products has not yet been determined.

The idea that asphaltogenic acids may form from oils after application to plants challenges the whole concept of chronic toxicity. One might legitimately ask: "How can it be proved that compounds originally present are responsible for chronic toxicity when time sufficient for asphaltogenic acid formation must be allowed for development of symptoms?"

There are two answers to this question. As revealed by the experiments with pure compounds, a series of substituted benzenes may show varying degrees of toxicity according to the extent of substitution. It does not seem probable that asphaltogenic acid formation would depend in the same way upon these substitutions.

A second, and probably sounder, answer is that relatively unrefined fractions such as Diesel oil and U.S. no. 1 fuel oil produce chronic toxicity and none of these unrefined fractions gained toxicity from standing in the light. They must, therefore, have contained the toxicants originally.

Oxidation of Oils. This discussion naturally evokes two questions: What is the nature of the acquired toxicity of oil fractions? Why is it acquired only by highly refined oils? Much research by oil chemists indicates that deterioration of lubricating oils and "staling" of gasoline and kerosene result from oxidation. Dornte (1936) showed that oxidation of white oils follows the general equation

$$V^2 = Kt + n$$

Where

V = total volume of oxygen absorbed (at normal temperature and pressure), cc per 100 grams of oil

t = time in hours

K = a constant that characterizes the rate of oxidation and

n = another constant related to inhibition of the reaction by foreign or natural inhibitors.

By plotting the square root of volume of oxygen absorbed against time, Dornte found that a straight-line relation is obtained having the slope K , with n as the point where the line intercepts the time axis.

When 100 p.p.m. of the antioxidant phenyl- α -naphthylamine was added, the value of n was about 20 hours. After this period, oxidation proceeded at

nearly the normal rate. The values of K (reaction rate) varied little with changes in partial pressure of the oxygen, being proportional to the $\frac{1}{4}$ power. At any time in the reaction about 25 per cent of the total oxygen absorbed was found as peroxide, 6 per cent appeared as water, and 6 per cent as acids.

From Dornte's results it is apparent that the oxidation of white oils is an autocatalytic process; that there is an induction period due to naturally occurring antioxidants; that one may lengthen this period by adding antioxidants; that the reaction proceeds at a nearly normal rate after the induction period has passed; and that the rate of the reaction varies little through a wide range of oxygen tensions.

Larsen and Armfield (1943), working with lubricating oils, found that oxidation stability of an oil is not achieved by removal of "unstable aromatics" (by refining), but rather that it results from the presence of natural antioxidants that stabilize the relatively reactive hydrocarbons constituting the bulk of such an oil. These results are confirmed by the work of Fenske and his co-workers (1941) and of von Fuchs and Diamond (1942). The effects of natural sulfur compounds in inhibiting oxidation of oil, as reported by Denison (1944), should be investigated from the standpoint of changes in toxicity to plants.

Evidently the aliphatic hydrocarbons are more reactive than has been assumed in the past; the removal of natural inhibitors by refining procedures brings about oxidation reactions that alter the chemical as well as the physical properties of an oil. Such reactions are apparently induced by light and temperature, and although oxygen is required, apparently certain changes (induction) may go on with only a limited supply present, leading up to a very rapid change when the oil is brought in contact with an unlimited supply. This later active stage may respond to metallic catalysts (Larsen and Armfield, 1943).

Tucker, in using highly refined high-boiling oils, was undoubtedly dealing with toxicity that resulted from oxidation. He was therefore justified in concluding that toxicity was due to asphaltogenic acids (oxidation products) and not unsaturates. It should be recognized, however, that at least three other types may occur as listed under paragraphs 1, 2, and 3 (p. 147 to 148).

On the Production of a Nontoxic Diluent. These considerations explain many of the results obtained in the experiments that have been described. The gains in toxicity by many of the refined oils were probably due to oxidation. The production of a nontoxic diluent therefore must involve two steps: first, production, by refining procedures or by synthesis, of a pure aliphatic oil; and second, the addition of a suitable antioxidant that is itself nontoxic at the concentration required. Furthermore, the time-temperature relation for the induction period for this product must be determined so that adequate storage conditions may be provided and the maximum "safe period" for using the oil determined. An antioxidant that would prolong the induction period for light catalysis of the oxidation reaction would improve such a diluent.

The natural occurrence, in oils, of antioxidants that protect against oxidation would seem to explain why stove-oil fractions did not gain toxicity upon storage in the light. Kerosene, gasoline, isoparaffin, and other highly refined products did gain in toxicity because they lacked these natural inhibitors.

The relation of the induction period and the small effect of oxygen tension upon the oxidation reaction of kerosene clarified the increase in toxicity during storage. The oil had stood in the dark exposed to the air for some time before being bottled and placed in the light. Undoubtedly it had become saturated with oxygen. When it was placed in the light, oxidation presumably started and carried on through the induction period. When the oil was applied to the plant (where it was spread in a thin film in contact with the air), the reaction undoubtedly speeded up, passing into the rapid phase with the production of increasing amounts of peroxides. Tucker (1936) showed that whenever the peroxide content exceeds 0.5 per cent, toxicity to apricot leaves becomes evident. Since oxidation may be autocatalytic, the rapid killing by these stored oil samples seems clearly to result from the products of this reaction.

Oil Selectivity. Another point of interest in the herbicidal use of oils is their selective action. As pointed out previously (Crafts, 1946), all selectivities of herbicides are relative, based on the dosage rate of the particular constituent to which the crop plant is resistant. Benzene, used pure for a sufficient exposure period, will kill grass, flax, and carrots; at a lower dosage rate it will kill grass and flax only; at a lower rate only grass. Gasoline will kill flax and grass; if diluted it injures only the grass. Kerosene, if fresh, will kill grass while causing no injury to flax and carrots. The compounds responsible for this injury are not selective; the natural selectivity of the fractions results from the particular dosage provided by each; not only concentration but exposure time as determined by volatility is involved.

Character of Oil Toxicity. Oil toxicity to many plants seems not to follow a linear relation but rather to involve threshold values. For instance, straight-run gasoline may contain around 20 to 30 per cent aromatics. At half strength it will not kill grass; at full strength it kills grass but not carrots; even at six times the normal dosage it will not injure carrots; apparently the moistening film will not hold enough toxicant to exceed the threshold of toxicity to these plants.

The threshold of toxicity involves not dosage alone but time and temperature also; apparently an expression of total chemical activity is involved. In cool weather, carrots will not be injured by stove oil with a gravity rating of 38° or higher at 100 gallons per acre; on a hot day such an application may cause some damage; similar damage may result when 150 gallons is used per acre on a cool day. Time, which affects exposure, enters the problem through the gravity of the oil. Gasoline at 150 or 300 gallons per acre will not injure carrots for it evaporates before injury can occur. If stove oil having a gravity rating of 34° is used, it will injure at 100 gallons per acre: it remains on the plants for many days, and, though its unsaturate content may be no higher than that of gasoline, it injures because of long exposure.

The exact nature of oil phytotoxicity is not well understood. The idea that tissues are killed by suffocation seems no longer tenable. In experiments recorded by Bailey (1930), cells were living after being surrounded with white medicinal oil for 500 hours. If suffocation were possible, these cells should have died.

If young active barley plants are sprayed with benzene, very soon an odor

similar to that of macerated leaves is discernible. Evidently, substances are released from the cells that ordinarily are freed only by complete destruction. Whereas the tissues will recover from a light spraying, prolonged exposure results in death. This would suggest that acute toxicity by unsaturates results from a rapid denaturing of the cell protoplast, a release of certain aromatic constituents, and finally complete disruption of the cells. The chlorophyll may become discolored within a few minutes in bright sunlight. Carrots give the same response, but somewhat more slowly.

Symptoms of chronic injury have been described on pages 146 to 148. They differ in many respects from those of acute injury; they involve the physiology as well as the biochemistry of the cells and tissues.

Toxicity due to oxidation of oils is different in nature from the type described above; it is less rapid than that from low-boiling aromatics, but very violent in its action. It is also nonselective at the dosage rates studied; apparently it results from a chemical mechanism involving qualities other than unsaturation.

Besides the applications that have been pointed out from place to place in this paper, the following suggestions have evolved in the study of oxidation. In research with oils it seems imperative that all samples be accurately labeled as to source, time of receipt, chemical nature, physical properties, and the like. Furthermore, if needed for future work these should be kept in dark bottles in a cool, dark place; and they should be tested periodically by some standardized procedure, not only for chemical changes but for attendant changes in toxicity.

If special oil fractions for spraying carrots, flax, or onions are produced will the addition of antioxidants protect them against increases in toxicity by oxidation? Where fuel fractions are widely used as general contact herbicides, might not their toxicity be considerably increased if oxidation could be induced? This result may prove particularly valuable if, in the future, improved fuels are freed of naturally occurring antioxidants by refining processes.

In producing spray oils for horticultural use, the manufacturer is faced with a dilemma. If little refining is used antioxidants will not be removed; but neither will unsaturates that are inherently toxic. If the oil is highly refined to remove all unsaturates, oxidation may render the oil toxic to foliage. Might not the answer to this problem be the production of highly refined oils of low unsaturate content and the addition to these of antioxidants—either natural, if they can be extracted without breaking down; or, if need be, synthetic?

Many other practical questions may be pointed out. Selectivity of oils, first used in killing weeds in carrot and celery crops, can be shown to function in flax and onion cultures in the greenhouse; barley seedlings have even been killed in cultures containing mustards and wild lettuce. Can this selectivity be carried to potatoes, cabbage, broccoli, cauliflower, beets, lettuce, and other truck crops in the field? These and many other questions present themselves for future study. They point out a fascinating field for research. Their answers will constitute a valuable body of knowledge for immediate application in practical agriculture.

SUMMARY

Oil Fractions. The tests on the relation of toxicity to boiling range have shown that two types of toxicity—acute and chronic—are caused by oils. Starting with the light fractions, acute toxicity increases with increased boiling temperature through the three light fractions of gasoline and reaches a maximum in the fourth fraction that boils between 330° and 420° F. Of the stove-oil fractions, acute toxicity is highest in the lightest, which boils between 320° and 390° F, but is practically equaled by the second fraction, boiling between 390° and 425° F.

Chronic toxicity begins to show up in the third stove-oil fraction and increases with increasing boiling temperature through fractions 2, 3, and 4 of Diesel oil. U.S. no. 1 fuel oil is high in chronic toxicity; but its high viscosity interferes with penetration, so that a mixture of 1 part with 3 parts of kerosene makes a more toxic material.

The refined fractions kerosene, odorless kerosene, and mineral seal oil are devoid of acute toxicity. All, however, show a certain degree of chronic toxicity, particularly against grasses. Mineral seal oil, the heaviest, was toxic to mixed weeds, grass, carrots, and flax by the seventh day of the test and more so as time went on. No one of the natural or synthetic fractions tested was entirely free of phytocidal effect.

Toxicity of fractional distillates of gasoline increases with weight of the fractions. Grass was killed by three heavy fractions and severely injured by the lightest. Onions recovered from injury by all fractions.

Cracked gasoline proved more toxic than straight-run.

Acid Treatment and Solvent Extraction. Acid treatment, by breaking down unsaturated compounds in petroleum fractions, reduces toxicity. Unsulfonated residues of stove oil produced by treatment with concentrated sulfuric acid were most toxic when made from the light cuts. Unsulfonated residues from heavy stove-oil fractions and Diesel-oil fractions were very low or completely lacking in toxicity. Carrots were not injured by unsulfonated residues from any of the oil fractions. The unsulfonated residue of heavy isoparaffin was nontoxic in the 8-day test period. In a 40-day period, unsulfonated residues from both stove oil and isoparaffin killed barley plants.

Unsulfonated residues of gasoline were more toxic than those of stove oil; those of cracked gasoline more than those of straight-run.

Solvent extraction removes the olefinic and aromatic compounds from petroleum fractions. Edeleanu extract and TS-28, a technical solvent prepared from this extract by fractional distillation, are both extremely toxic to carrots as well as to weeds. Avon Weed Killer, a residue remaining after the removal of valuable solvents from Edeleanu extract, is also very toxic. It is somewhat more toxic than the original extract to carrots, probably because it is heavier and thus more concentrated in chronic toxicants. When fractionated by distillation, the heavier cuts of both Edeleanu extract and Avon Weed Killer are more toxic.

When kerosene distillate is extracted repeatedly, the first extracts are most toxic. The final raffinate is like odorless kerosene in toxicity—that is, very low.

Coal-tar still residues are highly aromatic, hence very toxic. Refining of one

such material reduced somewhat the acute toxicity, but increased the chronic toxic properties.

An attempt to separate olefins and aromatics from a gasoline fraction by acid treatment resulted in greatly reduced toxicity but no clear separation of compounds. Detailed study of heavy olefinic and aromatic fractions proved the latter to be the more toxic and indicated them to be somewhat more selective than olefins between carrots and grass.

A naphthenic fraction proved intermediate in toxicity; a heavy isoparaffinic fraction nontoxic; and a fraction called naphthone A very toxic. A high-sulfur gasoline was very toxic if used without dilution, and *n*-cetane proved useful as a nontoxic diluent.

Toxicity and Boiling Range of Edeleanu Extract. When a series of temperature cuts of Edeleanu extract were tested for toxicity to plants, the heavier cuts invariably proved most toxic.

Storage and Refluxing. Gasoline samples gained in toxicity upon standing in the light in clear glass bottles. Refluxing of samples lowered their toxicity slightly; if air was passed through the apparatus during refluxing, the toxicity was a bit higher. Refluxing caused the samples to darken.

Upon standing in the light, samples of both kerosene and gasoline increased in toxicity; this toxicity was not selective on carrots. The theory is proposed that these changes result from formation of peroxides.

Pure Compounds. The toxicity of simple aromatic hydrocarbons increases with increasing substitutions in the benzene ring, as do their boiling points.

Xylene was equal in toxicity to gasoline, but not to a commercial aromatic solvent or stove oil. At the proper concentrations, xylene killed weeds in carrots selectively.

Tests on iso-, diiso-, triiso-, and tetraisopropylbenzenes proved that toxicity increases, in a general way, with increasing substitution. Both acute and chronic toxicity occurred. Since, however, the tests differed in detailed results, further work will be required for a clear picture of the relation.

Onions and carrots both tolerate the aromatic hydrocarbons.

Four chemically pure aliphatic hydrocarbons proved relatively nontoxic on fairly mature plants. These should prove useful as diluents in toxicity tests on aromatic and olefinic compounds.

Three saturated-ring carbon compounds proved toxic to plants.

Four mercaptans proved too low in toxicity to be of any herbicidal value.

In extended tests on *n*-hexane and *n*-cetane, the first caused acute injury, the second chronic injury, to young succulent flax and barley plants. Unsulfonated residues of stove oil and of the heavy isoparaffin that had been stored for several months in the light proved toxic, the latter to flax as well as barley.

Selectivity of Oils. Xylene will kill weeds selectively in carrots, with little or no harm to the latter. Onions and flax likewise manifest tolerance to the toxic effects of xylene. Grasses seem particularly susceptible to the toxic materials in oils.

Olefinic and aromatic compounds from oils also show selective action between weeds and the crop plants carrots and flax.

Onions tolerate both gasoline and stove oil. Fractions corresponding to the middle boiling range of gasoline apparently display the greatest selec-

tivity. Gasoline proved better than stove oil and Diesel oil for spraying onions in the field.

Weeds vary with respect to their reaction to oil sprays. Grasses are particularly susceptible to oil injury; mustards and fiddleneck are also easily destroyed; pigweeds, goosefoot, and lambs'-quarters are intermediate; sow thistle and wild lettuce are hard to kill; dense, matted chickweed is also difficult.

Kerosene, though initially low in toxicity, by slow chronic action kills grasses without injury to several crop plants. It may prove a valuable selective spray.

Physical Properties of Oil and Application Methods. Oils generally used for weed control have low viscosities and surface tensions. As compared with aqueous solutions they wet plants exceedingly well. They spread and creep downward; they penetrate the cuticle and come into intimate contact with the living protoplasm of cells.

Plants having spreading, open structure may be almost completely wet by aqueous sprays. Grasses are difficult to wet with water; but an oil, because of its physical properties, can wet their surfaces, creep into their crowns, and penetrate their cuticles. This may explain, in part, the specificity of oils for grasses.

Diesel oil sprayed on the top half of 10-inch barley plants ran down the leaves and stems and killed the crowns if the cultures were placed in a vertical position. Creeping occurred to a distance of only 2 inches if the cultures remained in a horizontal position.

When oils were applied to barley plants by the depositing of several drops in the funnel-formed top leaves, gasoline proved most toxic. When oils were applied as a spray to plants in a vertical position, kerosene was most toxic, followed by Diesel oil. The latter killed more slowly; but both agents effected complete kills. Applied to the crowns by means of a brush, gasoline and kerosene penetrated and killed the plants; Diesel oil caused injury but did not kill.

The properties inherent in Diesel oil as a result of refining make it almost ideal as a general-contact spray material. If improvement of this oil for Diesel-fuel purposes involves removal of olefinic and aromatic fractions, the herbicidal value will be reduced.

Stove oil as produced commercially meets very well the requirements for a selective oil in vegetable crops. A slight drop in its A.P.I. gravity rating or the removal of unsaturates would render this fraction less satisfactory.

In the future production of herbicidal oils, manufacturers should balance toxicity and spreading and penetrating powers in order to meet the requirements of particular weeds and crops.

Properties of Emulsions. Emulsifying of Diesel oil has not proved useful in weed control.

Stove-oil emulsions proved somewhat more toxic than straight stove-oil sprays of the same oil volume. Selectivity between carrots and weeds was reduced.

Emulsions of Avon Weed Killer and water were selective as between carrots and weeds. Stabilizing the emulsion with Vatsol increased the toxicity. The unstabilized emulsion failed to kill broad-leaved weeds.

Xylene and a technical solvent obtained by distillation of Edeleanu extract both proved selective against weeds in onions. Emulsions killed faster than straight oil sprays, but penetrated less well. Grasses recovered from injury by emulsions but died from oil sprays.

In tests on emulsions of normal Avon Weed Killer and of a type that forms an invert emulsion, no significant difference in toxicity was found.

Comparison of emulsions of Diesel oil and water, with and without the addition of sulfur, proved that sulfur increases appreciably the toxicity of such a mixture. Two grams of sulfur was roughly equivalent to 166 ml of Diesel oil. Plants sprayed with the emulsion containing sulfur became bleached during the second week after treatment.

Fortified Oils and Oil Emulsions. Acute toxicants that occur naturally in petroleum oils are of a low order of toxicity. Chronic toxicants are more effective but slower in their action.

Nitro- and chloro-substituted phenols carry high acute toxicity. They are valuable for fortifying oils of low acute or chronic toxicity.

The higher the content of aliphatic hydrocarbons in an oil, the poorer that oil's solvent properties for substituted phenols.

Among the dinitro-substituted phenols, the longer the substituted aliphatic chain the more soluble the compound is in oils.

In emulsions containing salts of substituted phenols in the aqueous phase or the parent compounds in the oil phase, toxicity increases with increasing oil content, but not always in a direct linear relation. When the oil content reaches a volume percentage of 6 or greater, killing by the emulsion approaches that of straight oil, provided the phenolic toxicant is above a limiting concentration. Where grasses mainly are concerned, an oil concentration up to 10 per cent is preferable.

Toxicity of oils may be greatly increased by fortification with phenolic toxicants. Such fortified oils may be applied direct at low volume rates, as by airplane; they may be extended by dilution with more oil; or they may be extended by emulsification with water. Preference depends largely upon the weed species being treated and upon relative costs of materials and application.

Oils also serve as carriers for 2,4-D. A low-toxicity kerosene has been used as a carrier for the methyl ester of 2,4-D on nut grass. Emulsions of Diesel or stove oils and 2,4-D salt solutions have been sprayed on cattails, tules, and bur-reed. Low-toxicity oils seem best as carriers of 2,4-D where this chemical is used on perennial weeds. Oil toxicity interferes with translocation of 2,4-D to the roots of such plants.

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THE BLACK GNATS OF CALIFORNIA¹

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TWO SPECIES of biting gnats of the family Ceratopogonidae (Diptera) occur in California. These are the valley black gnat, *Leptoconops torrens* Townsend, and the Bodega black gnat, *Holoconops kerteszi* Kieffer. They are both vicious biters and are frequently extremely annoying to man, livestock, and poultry. Although the adults are well known to all residents of the breeding areas, the immature stages—egg, larva, and pupa—have remained undiscovered up to the present time. This study was undertaken to discover these immature forms, to determine the breeding areas, and to provide as much biologic information as possible, as a basis for control.

CLASSIFICATION

There are four genera of closely related flies—*Styloconops*, *Leptoconops*, *Holoconops*, and *Microconops*—which differ markedly from all other genera of the family Ceratopogonidae. All authorities recognize this distinct group of genera as the *Leptoconops* group. Enderlein (1936)⁴ established the subfamily Leptoconopinæ to contain these genera. Johannsen (1943) distinguished the adults of the *Leptoconops* group as having 12 to 14 antennal segments and no radiomedial cross vein, whereas the other ceratopogonids have 15 antennal segments and possess the cross vein.

The larvae of *Leptoconops* and *Holoconops*, described later in this paper, show such marked differences from the usual ceratopogonid type of larva that the writers agree with Enderlein and accept the subfamily name Leptoconopinæ for this group. The chief characters in which the larvae differ from the other ceratopogonids are: the presence of heavy mandibular rods which extend backward into the first or second thoracic segment; the presence of 21 or more body segments; and a great reduction in the pharyngeal skeleton, with the pharyngeal combs entirely absent.

Only two genera of the Leptoconopinæ are known to occur in California. These are the genus *Leptoconops* Skuse, in which the females have 14 segments in the antennae; and the genus *Holoconops* Kieffer, in which the females have 13 segments in the antennae. The females of the valley black gnat, *Lepto-*

¹ Received for publication March 8, 1947.

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⁴ See "Literature Cited" for citations, referred to in the text by author and date.

conops torrens, were named and described by Townsend (1893) from specimens collected near Patterson, New Mexico, June 21, 1892. The females of the Bodega black gnat, *Holoconops kerteszi*, were named and described by Kieffer (1908) from specimens collected at Cairo, Egypt. The males of both species were described by Freeborn and Zimmerman (1934) from specimens collected at Davis and Bodega, California.

DISTRIBUTION

The black gnats are widely distributed throughout the world but occur only in very limited discontinuous areas where suitable larval habitats are found. The Bodega black gnat has been recorded in Egypt by Wiess (1912), at Cairo, Behera, Wadi, Natroun, and Sakkara. In Tunis it was taken at Tabeditt. In North America it has been recorded by Carter (1921) in the vicinity of the Great Salt Lake, Utah, and by Freeborn and Zimmerman (1934) at Bodega Bay, California. The writers found it at Dillons Beach, a resort town near the town of Tomales, California.

The valley black gnat is recorded by Freeborn and Zimmerman (1934) at Uvalde and Dallas, Texas; Las Vegas, Nevada; and in Yolo County, California. Johannsen (1943) records it in New Mexico and Colorado. The writers have collected this species along the western side of the Sacramento Valley from Colusa to Suisun, at Cupertino in the Santa Clara Valley, and at Merced and Tulare Lake in the San Joaquin Valley. There are no records of this species outside of the United States, although a few species of this genus are known in South America.

DESCRIPTION OF THE STAGES OF THE BODEGA BLACK GNAT

The Egg. The egg (fig. 1) is white when first laid, and slowly turns to very dark brown as it matures. The eggshell is unsculptured, but the contents of the mature egg give the illusion of a reticulation on the surface. The length of the egg is 0.34 mm; the width, 0.10 mm. The shape varies slightly in different eggs, but most of them are banana-shaped with one end slightly narrower than the other. They vary from straight to slightly curved and are circular in cross section.

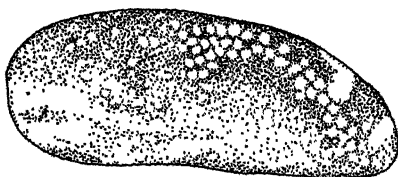


Fig. 1. Egg of the Bodega black gnat.

The Larva. There are four larval instars, alike in all fundamental features except size. The length of the first-instar larva upon hatching is 0.50 mm and the width is 0.13 mm. The second-instar larvae vary in length from 1.5 mm to 2.5 mm, the third from 2.5 mm to 4.2 mm, and the fourth from 4.2 mm to 5.6 mm. The head is thinly chitinized with localized chitinous thickening. Within the head region and extending into the first thoracic segment there is a system of heavily chitinized rods connected with the mandibular processes (figs. 2 and 3). The dorsal process consists of a median rod extending anteriorly for approximately four fifths of the head segment. At its posterior end there is a transverse bar just inside the second segment. It consists of three sections. On the outer extremities of the crossbar

are two additional longitudinal bars, the dorsolateral rods, which extend from the posterior third of the first segment, two thirds of the way into the second segment. These two dorsolateral rods, by gradually sloping to the ventral surface, contact the two ventrolateral rods, which are the heaviest in the system. The two ventrolateral rods extend from the point of contact with the dorsolateral rods forward to short mandibular levers, which are in turn attached to the mandibles. The mandibles are swung by this whole ventral process and brush the food onto a scooplike labrum.

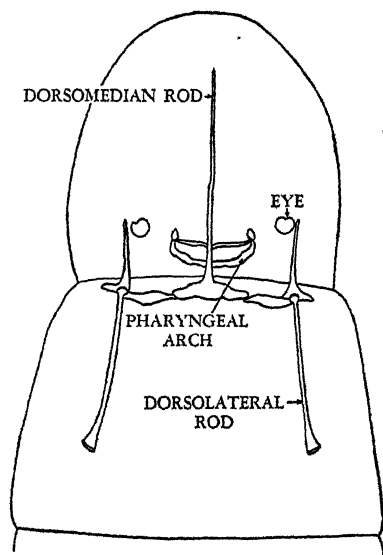


Fig. 2. Head and first thoracic segment of the Bodega black gnat, dorsal view, showing chitinated structures in the dorsal part of the larva.

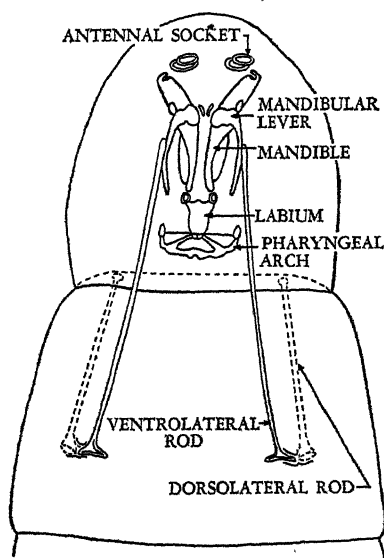


Fig. 3. Head and first thoracic segment of the Bodega black gnat, dorsal view, showing chitinated structures in the lower half of the larva.

Immediately anterior to the mandibles are two bristles which may be easily seen when the mandibles are in motion. At the extreme tip of the head segment are found the antennae, which may be extended or withdrawn into the head so that they appear as two small caps.

The eyes are round and black. They are located between the dorsolateral rods and the ventrolateral bars about three fourths of the way from the anterior end of the first segment.

The thoracic segments have no special characteristics, but each abdominal segment (except the anal) has an intercalary segment, giving the larva the appearance of having twenty-three segments in all. The anal segment terminates in three lobes. The larvae are without spines, hairs, or bristles except for the two located just anterior to the mandibles. Larvae in the first two stages are colorless and transparent, but as they reach the third instar they become orange in color.

Female Pupa. The length of the female pupa (fig. 4) is 2.7 mm and the width 0.6 mm. (measurements taken on preserved specimen). The length of the

respiratory trumpet is 0.13 mm. The thorax is very dark brown, the abdomen varies from tan to dull orange. The respiratory tube has two segments. The anterior segment is oval, with the broadest part, possessing fifteen transparent spiracles, located apically. The head is rugose, without armature, other than

spines. The antennae extend from a region above the eyes to a region just beyond the middle of the wing pads. The wing pads appear to be crumpled. The legs lie along the anterior border of the wing pads. The first pair and a part of the second appear between the two wing pads, but the third pair is not visible except for the tips, which lie between the tips of the second pair of legs and the most posterior portion of the wings. The segments of the abdomen are visible from the dorsal side; all segments except number ten are armed with sharp recurved spines. These spines are all set on large tubercles and have a very characteristic arrangement. Segments one to seven have two spines in a row anteriorly, and four in a row posteriorly, on both dorsal and ventral sides. On each lateral border of these segments there are two spines. This makes a total of sixteen spines for each segment. Segment eight has only two spines on the dorsal and ventral surface and two on each lateral margin, a total of eight spines for this segment. The ninth segment has two spines, one on each lateral border. There are two tubercles dorsally and ventrally in the same position as the spines of the eighth segment. The last segment is forked for about one half of its length, and is without spines or tubercles.

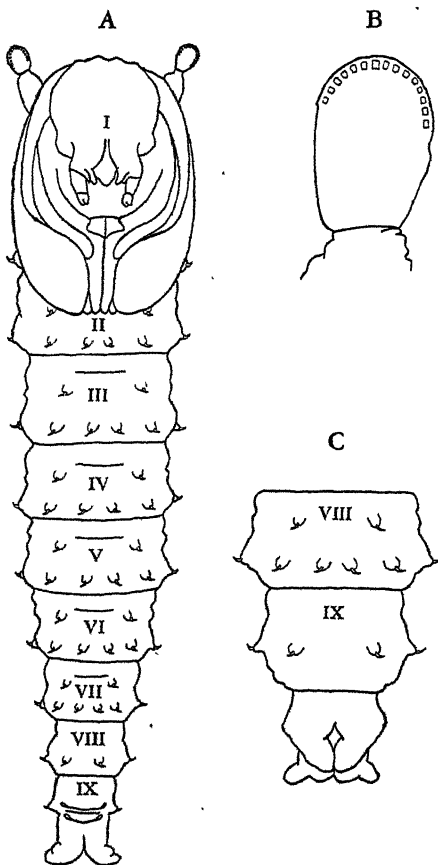


Fig. 4. *A*, pupa of female, Bodega black gnat, ventral view; *B*, respiratory trumpet of the pupa; *C*, posterior three segments of the abdomen of the male pupa, ventral view.

Male Pupa. The male pupa (fig. 4, *C*) is similar to the female, but has a thicker antennal sheath and a different arrangement of spines on the eighth and ninth segments. The arrangement of spines is the same on the eighth segment as it is on the preceding seven segments. The ninth segment has the same arrangement of spines as the eighth segment of the female. The tenth segment terminates in a pair of lateral wings which encase the basistyles and the distostyles of the adult terminalia.

Adult Female. The length is 2.5 mm to 3.0 mm. When seen in the field the adult gnat seems black, but when cleared in Berlese fluid and examined under

a microscope it shows many light-brown portions and the very dark areas are dark brown. The head and thorax are very dark brown; the abdomen is much lighter. The abdomen is also lighter ventrally than dorsally. The wings (fig. 5) are transparent but when folded on the back and seen by reflected light, appear opaque white. The legs are dark brown at the base, becoming lighter at the extremities.

The color of the head is very dark brown. The compound eyes are reniform and have no special characteristics. The eyes are separated in front by a rather large space in which the two large, globular antennal sockets are set.

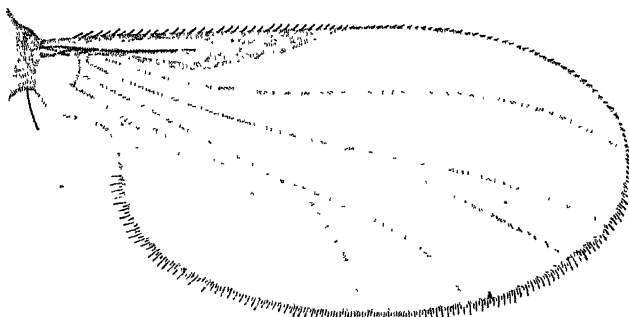


Fig. 5. Wing of female, Bodega black gnat.

The antennae (fig. 6) are dark brown and are made up of thirteen segments. The basal segment is flat and ringlike. There are three bristles on the inner margin of this segment. The second segment is subspherical and has a deep depression on its anterior face for the reception of the third segment. It has a general covering of very fine hairs. The third segment is pyriform. It is about half as large as the second segment and slightly larger than each of the following nine segments. On the anterior third of this segment there is a whorl of six large, erect bristles. The segment has a general covering of very fine hairs arranged in rows. It is much more sparsely covered than the second segment.

Segments four to twelve are globular and have a moniliform appearance. Each segment has a narrow, pale, basilar section, inserted like a pedicle into the circular pit of the anterior portion of the preceding segment. The body of each segment is dark brown and somewhat broader than long. Each segment has a whorl of ten rigid, dark-brown bristles around its center. The bristles are long and stout. There is on each of these segments a fine covering of small hairs arranged in rows, similar to the hair covering described for the second and third segments. These hairs are arranged more sparsely than those on the third segment.

The thirteenth segment has about the same diameter as the preceding nine, but is about three times as long. Just above the base it has the whorl of ten bristles which characterizes the preceding segments. Following these is a series of imperfect whorls. The segment ends in a terminal bristle almost at the apex of the segment.

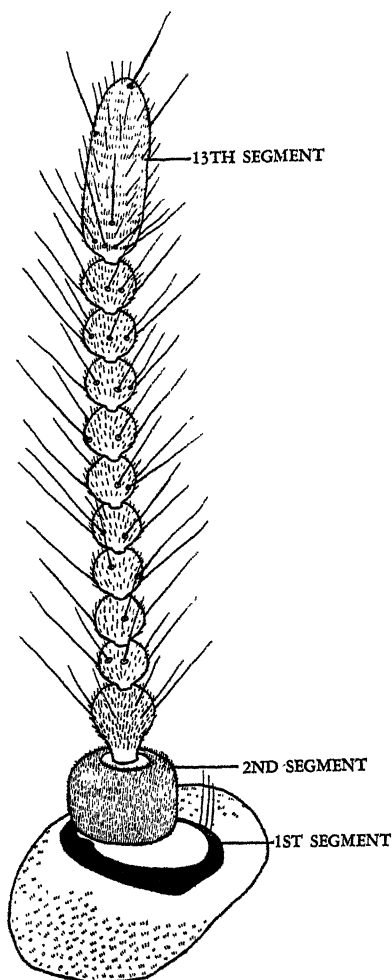


Fig. 6. Antenna of female, Bodega black gnat.

The third segment is very dark brown, pyriform, and slightly constricted at the middle. It is inserted into a circular depression of the second segment by a narrow pedicle. On the surface nearest the maxilla, in the distal two thirds of the segment, is a deep cavity, about half as long as the segment itself. There is a dark chitinous ring around this cavity, and within the cavity are several platelike sense organs.

The fourth segment is white at its base and dark brown at its tip. There is a terminal bristle and a whorl of six bristles just below the tip. There are numerous whorls of smaller bristles in rather even rows for the complete length of the segment. The fourth segment is about twice as long as the third, but only one third as wide. These four segments of the palpus are shown at the right in figure 7.

The mouth parts (fig. 7) consist of a labrum, labium, hypopharynx, two mandibles, and two maxillae with their maxillary palpi. The length of the proboscis is $210\ \mu$. The labium does not completely encompass the rest of the mouth parts; the pilosity is not particularly distinctive; the bristles, fine and coarse, which are similar to those that cover the whole body of the insect, are very light in this region. They are most numerous on the labella. The labrum is slightly dilated at its base and bears at its apex apparatus for perforating the skin. This consists of two terminal toothlike structures of a type similar to those found in the genus *Culicoides*, family Ceratopogonidae. The hypopharynx is lanceolate, light in color and soft, but thickens toward the median line. The mandibles are pale, slender structures with ten large, sharp teeth at the apical end to aid in cutting tissues of the host. The maxillae are very light brown in color and are wider and thicker than the mandibles. The number of teeth varies from sixteen to nineteen. The teeth of the maxillae are smaller than those on the mandibles and are set closer together. The teeth of the mandibles are directed outward, the teeth of the maxillae inward.

The palpi are composed of four segments. The basal segment is short and light-colored. The second segment is light and a light-brown area covers most of the distal end, which has a whorl of four large

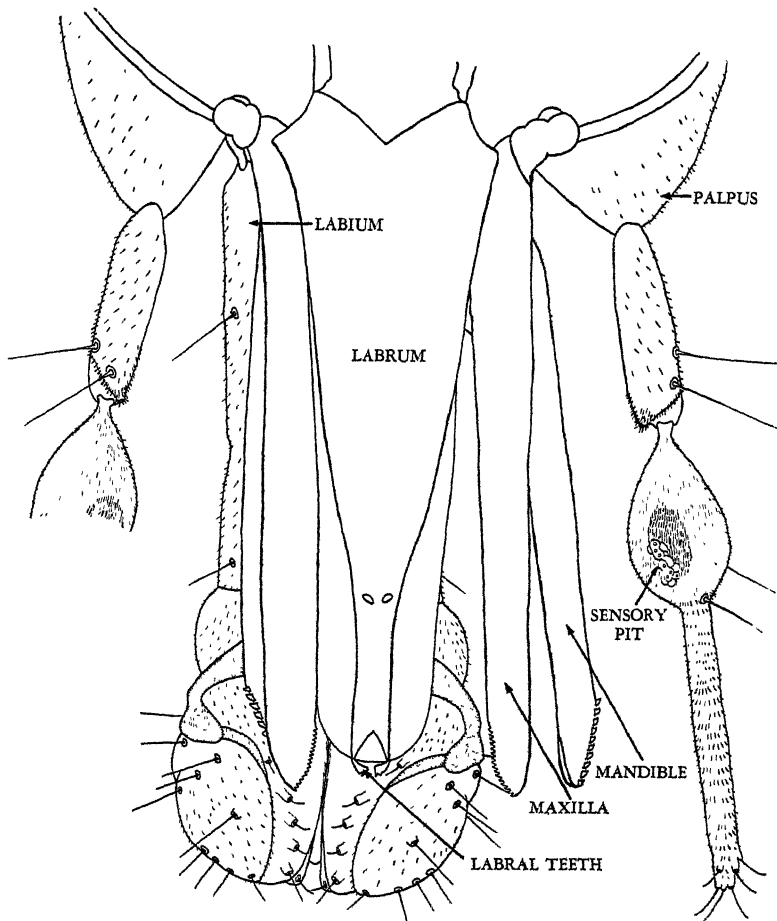


Fig. 7. Mouth parts of female, Bodega black gnat.

The thorax is strongly arched. The prothorax is indicated by two gibbous structures, located dorsally and ventrally. The scutellum and metanotum are small, and the scutellum has four very stout bristles directed caudad. There are three longitudinal rows of stout bristles on the dorsal side of the thorax. Laterally there is a group of sixteen irregularly arranged bristles on each side. The sensory organs in the humeral region are conspicuous and crescentic in shape. Dark brown in color, they are located just ventral to the base of the wing.

The length of the wing (fig. 5) is 1.65 mm and the breadth 0.70 mm. The wings are covered with numerous very small hairs. The halteres are oval and white. The anterior margin of the wing has a row of very closely placed recurved hairs, slightly larger than the small hairs which clothe the general wing surface. The hairs become larger gradually as they approach the distal portion of the wing and are largest on the posterior margin. The arrangement

of hairs on the posterior margin is very characteristic: there is an almost perfect alternation of long and short hairs. It is possible that where the arrangement is not perfect, bristles may have been lost.

The costal vein does not reach the middle of the anterior margin of the wing, but stops approximately at the bifurcation of the fifth longitudinal

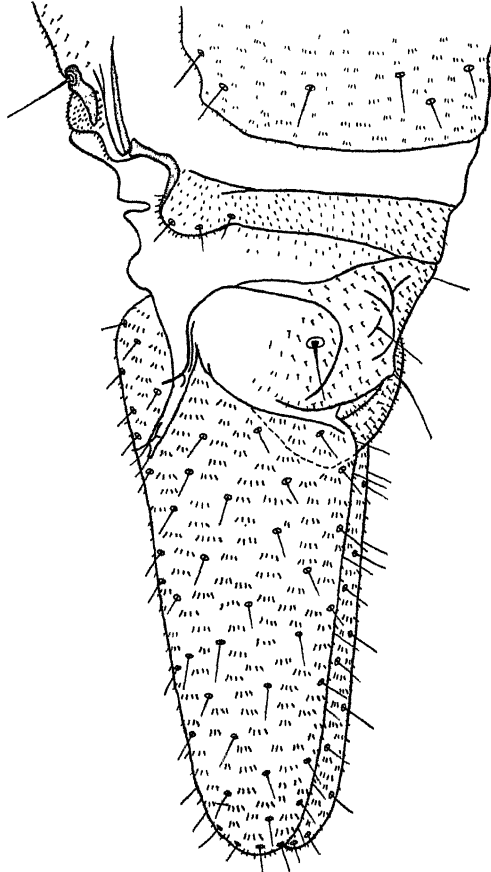


Fig. 8. Terminalia of female, Bodega black gnat.

vein. A wide, dark zone results from the fusion of the costal and subcostal (or first longitudinal) veins, an area which is thus strongly reinforced. There is no trace of the second longitudinal vein, which, however, might be in the confused mass of the united costa and subcosta. The third longitudinal vein is arched and describes a curve parallel to the anterior border. It does not reach the border, falling short almost at the tip.

The fourth longitudinal is approximately rectilinear. The upper branch goes from the axis of the wing to the apex. The basal portion of the lower branch is absent, and the distal end does not reach the apex of the wing. The fifth longitudinal vein is the thickest of the longitudinal veins, and is divided into arched branches which reach the posterior border at its middle part. The

sixth and seventh longitudinal veins are represented by two weak rectilinear veins. In summary: aside from the mass formed by the costa and subcosta, there are three well-defined longitudinal veins, two of which are bifurcate. There are apparently no cross veins. Langeron (1913), after examining the literature on wing structure of this species, came to the same conclusion, suggesting that if cross veins do exist, they must be located at the extreme wing base, and are, consequently, very difficult to see.

The stigma was described by Austin (1921) as "large and very conspicuous, and of a striking orange color." His description was made from specimens taken in Tunis. He did not state whether the specimens were male or female. Freeborn and Zimmerman (1934) described the stigma of the female as brilliant scarlet, but stated also that the color was not constant, varying from

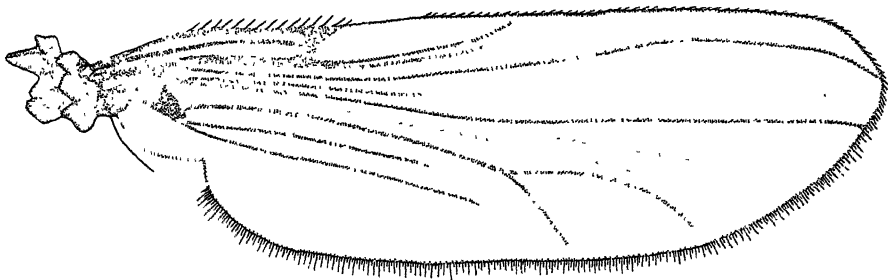


Fig. 9. Wing of male, Bodega black gnat.

orange red through pale yellow to practically colorless. Their descriptions were of specimens taken at Bodega Bay, California. Almost all specimens taken at Bodega Bay by the authors had colorless stigmata. A few females taken at the end of the adult season in October, 1944, and a single female taken in March, 1945, had brilliant orange red stigmata.

The abdomen bears two lamellae (fig. 8) which are attached to the sides of the tenth segment. The lamellae are approximately four and one half times the width of the tenth abdominal segment. They are covered with very small hairs arranged in groups of three or four in a line. There are also some larger hairs dispersed evenly over the entire structure. A smaller pair of lateral structures are attached to the ninth sternite and extend over one fifth of the lamellae. There are two heavily chitinized spermathecae, oval in shape. These are seen in the seventh segment.

Adult Male. The male is darker than the female and is somewhat larger. The length of the male is 3.5 mm to 4.0 mm, the width at the widest region of the thorax, 0.7 mm. The head and thorax are very dark brown; the abdomen is much lighter. Live specimens appear shiny black when taken in the field. The abdomen is slightly lighter dorsally than ventrally. The wings (fig. 9) are clear or opaque white as in the female, and are covered with very small hairs. The legs are dark brown at the base, becoming somewhat lighter distally.

In general the head is very dark brown. The appendages of the head other than antennae and mouth parts are the same as those of the female.

The antennae of the male are fifteen-segmented. The first segment is ring-like and clear, with a heavily chitinized circle at its base. The second segment

is very much enlarged and, when seen from above, appears to be doughnut-shaped. It is clothed with very small hairs. The third segment is pyriform as it is in the female antennae. The fourth to the fourteenth segments are very similar, but become progressively narrower; each is three times as long as its widest portion, and has a swollen ring to which is attached a whorl of very long bristles. Segment fifteen is slightly over twice the length of segment fourteen and is about the same width except at the tip, which is about twice

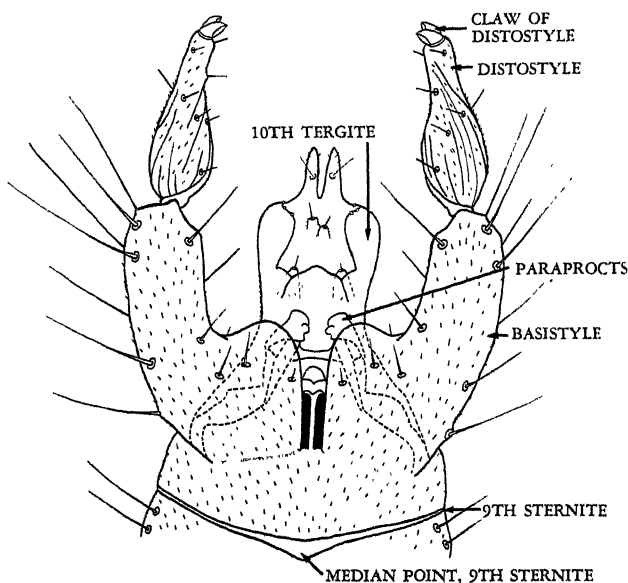


Fig. 10. Terminalia of male, Bodega black gnat.

as broad as the narrowest portion. At the base of the fifteenth segment there is a whorl of the long bristles which are characteristic of all segments from the third to the fifteenth. These give the antennae their plumelike appearance. There are many small hairs and sensory pits located on the fourteenth and fifteenth segments.

The mouth parts are similar to those of the female, but are more slender and are not adapted to the blood-sucking habit of the female. They appear to be strong enough to pierce plant tissue or thin insect integument. The labellae are fleshy and prominent, but thinner than in the female. The palpi have four segments. The basal segment is fleshy, light, and less than half as long as the second. The second segment is light-colored, long and narrow, becoming broader distally. The third segment is slightly swollen two thirds of the way toward its distal end, and long, slender bristles are attached to the distended portion. This segment is dark brown and has an inconspicuous sensory pit. The fourth segment is about the same length as the third segment, narrow, swollen distally, but narrowing again at the apex. The proximal one third of the segment is white, the distal two thirds dark brown. All segments are clothed with very short hairs.

The mandibles and maxillae are more slender than the corresponding structures in the female. The teeth are more delicate and much longer. The labrum has a whorl of long hairs at its tip instead of the two toothlike structures of the female.

The wings of the male (fig. 9) are longer and narrower than the wings of the female.

The abdomen is longer and narrower than the abdomen of the female.

The terminalia (fig. 10) of the male are important in taxonomy. The basistyles are short and stout. There are large basal lobes and apical condyles for articulation with the distostyle. The distostyles are wide basally, becoming narrower toward the distal end, and terminating in two spoonlike structures. There are two median digits located at the distal end of the ninth tergite. The lateral wings of these structures are bent ventrally to form a partially closed cylinder, in which may be seen a fleshy mass terminating in four lobes. This is probably the tenth or anal segment. These lobes are covered with setae. The aedeagus consists of two simple valves. The paraprocts are heavily chitinized structures which originate at the base of the basistyles. The ninth sternite is very narrow, and has a small median point.

DESCRIPTION OF THE STAGES OF THE VALLEY BLACK GNAT

The valley black gnat closely resembles the Bodega black gnat. The points of difference between the two will suffice to describe the valley black gnat, and, at the same time, serve to differentiate the immature stages of these two species. The pupal stage has not been obtained. Eggs were secured only by dissection of gravid females and agreed in all respects with the eggs of the Bodega black gnat.

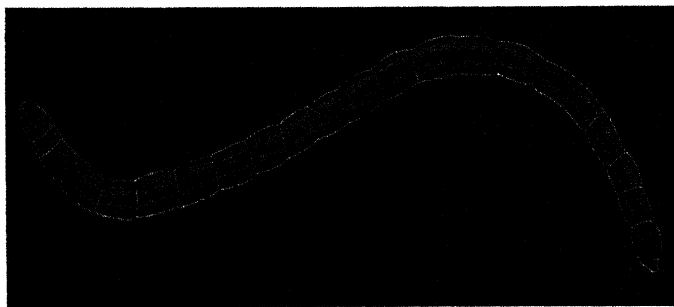


Fig. 11. Larva of the valley black gnat as seen in life by reflected light.

The Larva. Larvae of all sizes have a distinct, slightly chitinized, transparent head and 21 body segments (fig. 11). The prothoracic segment is only half as long as the other body segments, which (with the exception of the last segment) are uniform in length and appearance. The intercalary segments cannot be differentiated from the true segments. They have probably arisen by the equal division of abdominal segments 1 to 9 inclusive. The last abdominal segment terminates in three blunt lobes (fig. 12), one dorsal and two

ventral, with the anus recessed between the lobes. Two ovoidal, thinly chitinized, transparent bladders (gills?) are protruded from the anal opening when the larvae are living in water. The larvae are apneustic, but have well developed tracheal systems. There is a very minute bifurcated bristle on the head between the antennal sockets, but otherwise the larva is without hairs or spines of any kind. The integument of the anal lobes is slightly rugose. Mature larvae average 5.35 mm in length, and a few extra large specimens measure 6 mm.

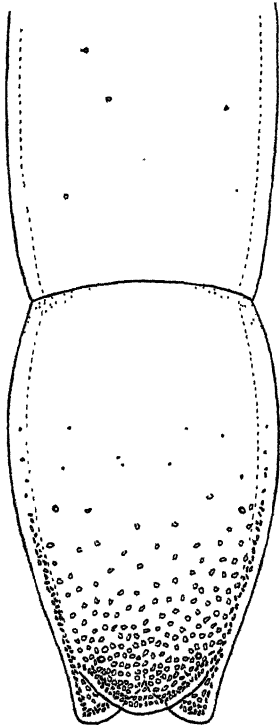


Fig. 12. Last segment of larva of the valley black gnat, dorsal view.

The antennae are one-segmented with a retractile bladder at the tip. The whole antennae is retractible into the head. Eyes are absent; no vestige remains. The color of living larvae as seen by the naked eye is a dense, opaque white, but when studied microscopically, alive in water, the fat-body is seen to be the structure which gives rise to the white appearance; the integument and internal organs are either transparent or translucent and are colorless.

The heavily chitinized mouth parts (figs. 13 and 14), which are easily visible through the head capsule, are the most conspicuous feature of the larvae. They are much more heavily chitinized in the valley black gnat than in the other species. They consist of a pair of concave, tridentate mandibles, so placed—with convex sides apposed—as to preclude biting; a pair of long chitinous rods, the ventrolateral rods, extend into the second thoracic segment. The mandibles are articulated to the rods by strong, heavily chitinized irregular pieces, the mandibular levers. The dorsolateral rods which occur in the Bodega black gnat are not found in the valley black gnat.

There are two vertical pharyngeal arches placed close together near the rear of the head capsule. The anterior arch is continuous with a horizontal U-shaped piece which lies in the floor of the pharynx. These arches appear to be the atrophied remnants of a masticating organ.

A heavily chitinized rod, the dorsomedian rod, extends about two thirds the length of the head. At its posterior end it bifurcates into a broad U; at the anterior end it is cleft and carries thinly chitinized lateral wings.

The antennal arches are forked structures located on either side of the head in such a position that the antennae can be retracted into the arms of the arch. These heavily chitinized arches are probably for the attachment of muscles which retract the antennae.

The labrum has two depressions in its anterior margin, each of which accommodates one mandible as it sweeps food into the mouth. The anterior edge of the labrum is heavily chitinized and is continuous with a thinly chitinous structure which extends posteriorly for about half the length of the head.

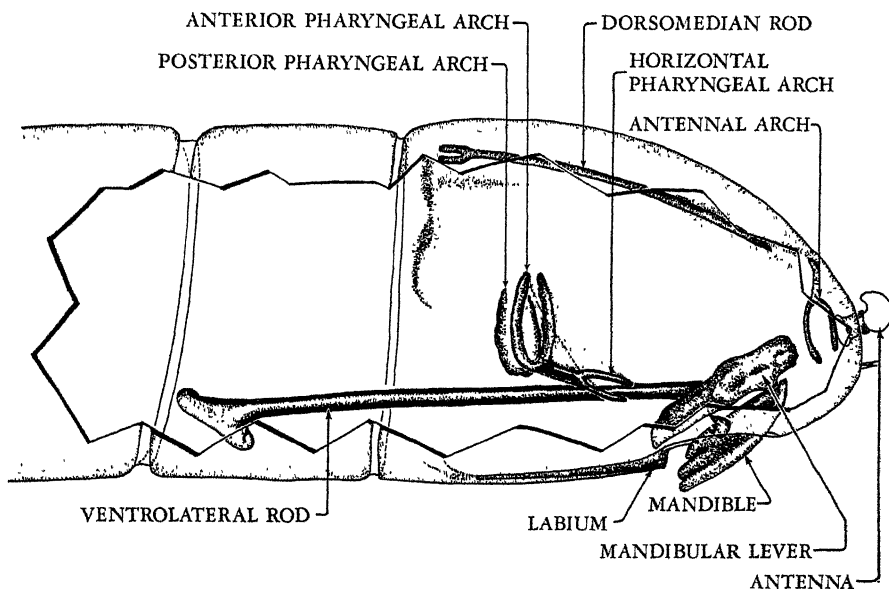


Fig. 13. Valley black gnat larva: cutaway diagram of head and two thoracic segments, with mouth parts and antennal parts removed from the right side of the larva.

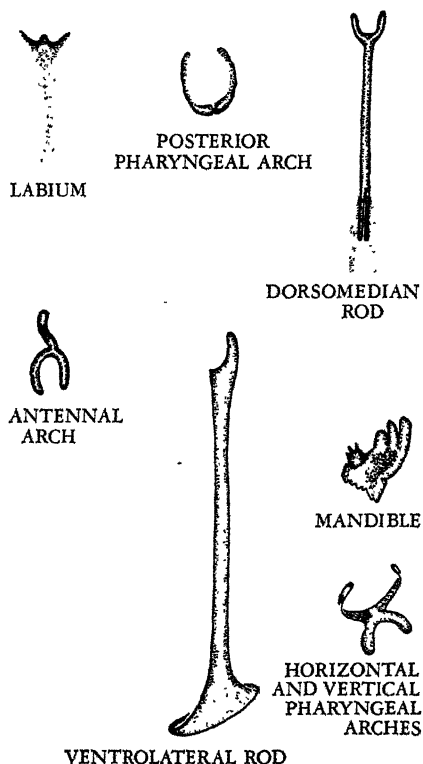


Fig. 14. Chitinized structures from the head of the larva of the valley black gnat.

There are some irregular, heavily chitinized areas of the integument in the posterior dorsum of the head capsule.

The Adult. The female of the valley black gnat closely resembles the female of the Bodega black gnat. The chief character which distinguishes these two species is the number of segments of the antennae—14 segments in the valley black gnat and 13 segments in the Bodega black gnat.

The male of the valley black gnat is slightly smaller than the male of the Bodega black gnat. The only distinguishing characters are found in the terminalia. For a description and illustrations of these characters see Freeborn and Zimmerman (1934).

BIOLOGY OF THE BODEGA BLACK GNAT

Breeding Grounds. The larvae of this species were found at Bodega Bay, living in damp sand along the margins of pools situated in rain gullies, just above the normal high-tide level. Salinity tests were made of the soil in which the gnats were breeding; the method used was that described by Mohr (in Kolthoff and Sandell, 1936), wherein chloride ion is determined volumetrically by precipitation with standard silver nitrate solution using potassium chromate as an indicator. The samples taken in Bodega Bay showed 8,900 p.p.m. Water sampled at the tide pool where the larvae were found showed 640 p.p.m. This sample was taken in March, when there was a considerable amount of rain water in the ditch. No larvae were taken in areas totally free of the chlorides.

In a region 10 yards nearer the bay the salt content was 1,300 p.p.m. No larvae were found here, although conditions of soil and plant life were similar to the area of lesser chloride concentration, where larvae and pupae were found in abundance. The larvae have a preference for brackish water, largely rain water and drainage, with only an occasional infiltration of tidewater. The breeding grounds where the larvae were taken in the greatest numbers were reached only by high tides of 6 feet or over. These tides occurred for a period of 10 days in January, 4 in February, none in March, 2 in April, 5 in May, 4 in June, 11 in July, 9 in August, 1 in September, 5 in October, 6 in November, and 7 in December, making a total of 64 days throughout the year when the breeding grounds were inundated with water of very high saline content. The water from these tides leaves the area fairly rapidly, but the sandy nature of the soil causes the water to penetrate deeply; the organic matter acts as a blotter to hold the moisture and maintain the soil in a semi-saturated condition favorable to the development of the larvae. The area where the larvae are found extends not more than 2 feet from the water's edge. During the winter months there is standing rain water, but in the summer the only added water is tidal. In summer the area of standing water is covered with vegetation. The larvae are not found in the area of standing water; the actual breeding area extends from the edge of the standing water to the end of the moist sand. This area will vary in width according to the slope—the greater the slope the narrower the breeding area.

The soil in which the larvae were found was the "nearly pure sand" referred to by Painter (1926) in his description of the soil in which *Holoconops bequaerti* larvae were found. This description contributed toward the finding

of larvae of *H. kerteszi*, as did his suggestion that breeding grounds occur at the high-tide mark. It was possible to eliminate many areas by confining the search to "nearly pure sand at the high tide mark."

The sand was tested for organic matter by igniting air-dry samples. Samples from the top 2 inches had 11.0 per cent organic matter, samples 2 to 4 inches deep, 2.8 per cent. The organic matter consisted chiefly of decaying plant material. There were many insect parts, such as egg cases, larval and pupal exuviae, and dead adults. The sand was rather dark, and it became reddish upon heating, suggesting the presence of iron compounds.

Methods. Infested sand was placed in a large glass funnel which was set in shallow water. A piece of fine cheesecloth over the stem of the funnel retained the sand. A 60-watt lamp was placed close to the top of the funnel. As the top layer dried out, it was carefully scraped off and thrown away. Gradually all the soil dried and the larvae fell to the bottom of the dish of water. Larvae had no difficulty in passing through the cheesecloth at the bottom of the funnel.

This method failed to yield eggs or pupae, but it was very thorough in removing larvae from the sand and was used to make accurate counts of population density. The method likewise yielded first-instar larvae, which could not be collected by the elutriation method.

The elutriation method was by far the most convenient and satisfactory way of collecting the larvae. A 1-pint jar was filled with soil to be tested and the jar placed in a pan with a white bottom. The ideal pan for this use was a photographic developing tray for 8 x 10 prints. The jar was placed under a faucet with a moderate flow of water, and the soil was stirred from time to time so that all small particles and organic matter were whirled by the force of the water and overflowed from the jar to the pan and then from the pan to the drain. After a period of 10 minutes all larvae had floated out of the jar and into the pan. The heavier particles of sand remained in the jar, which was removed from the pan. All excess water was decanted from the sample in the pan, and this prepared sample was then taken into sunlight where the larger larvae were removed. A gentle rotary motion of the pan separated the larvae from the organic matter which had also floated out into the pan. The larvae were easily recognized by their tendency to curl up like a doughnut. They were pipetted into watch glasses, together with a small amount of organic material; they remained in the watch glasses until they pupated and emerged. They showed no cannibalistic tendencies, and it was possible to keep as many as forty or fifty in a single watch glass.

The smaller larvae and eggs were recovered only by an examination of the organic matter remaining after the larger larvae had been removed. This examination was made under a binocular at 15 magnifications, the organic matter being placed in small lots in a white saucer. The eggs were washed to the edges of the saucer by a rotary movement similar to that used for the recovery of the large larvae.

The pupae were easily seen and separated out with the large larvae. Not all of the pupae were removed by the differential elutriation method because they were too heavy to be washed out of the jar. However, they were clearly visible at the top of the soil remaining in the jar.

Seasonal Cycle. The males in the field congregate in large groups of several hundred to a thousand. The clusters they form vary in size from a cubic foot to 2 cubic yards and, when netted, are found to include few females. The females, which may be numerous in the vicinity, may visit the male swarms, probably for copulation. The females taken in swarms of males were crushed and found to contain no vertebrate blood, so it was assumed that the blood meal was taken after copulation.

Human blood is not the only blood taken by the females. Dogs and cats are probably preferred hosts. When feeding on a dog or cat, the gnat may hide in the thick fur and take a blood meal undisturbed, since these animals are not irritated by the gnat bites and do not develop swellings such as appear on man.

The first large swarms of adults at Bodega Bay occurred in mid-April. Three sweeps of a standard insect net resulted in a collection of 144 males and 3 females. Residents of the area complained that the gnats were biting fiercely every warm day. The pools were without surface water for the first time since December. The sand was moist and conditions appeared to be right for egg laying.

Large swarms of dancing males, together with some females, have been observed 575 yards southeast of the breeding area, and at an elevation of 100 feet above the breeding area. The prevailing winds are northwest. Certain definite locations are selected for swarming, and swarms of males may be found in those areas on all calm, warm days, year after year. The males appear to select a lee, and fly close to, but not into, the streaming air which comes around one side of the windbreak.

Attempts were made to determine the total length of life of adult male and female gnats, but, since the experiments were made on field specimens, the evidence is inconclusive. Under a close approximation of salt-marsh conditions, all the males died within 4 days. They had been taken from a breeding swarm in the field and, therefore, the total length of life cannot be known. The females, which were kept in cheese glasses with a single layer of moist blotter paper at the bottom of the glass, were fed as often as they would take a blood meal. They took as many as 4 meals and lived, in some cases, as long as 11 days. Since these were also field specimens, it is possible that the total life span is considerably longer.

The first eggs were taken from the soil on June 11, and on June 13 two of them hatched. The remaining 18 eggs hatched June 23. This indicates that the incubation period is at least 12 days. Since the eggs and mud were thoroughly stirred up by the method of egg collecting described above, it was impossible to tell whether the eggs were laid singly or in a group. However, it is thought that the eggs are laid singly, since none were ever collected in a mass or even in pairs. The eggs are very light tan at first, gradually darkening as they reach maturity. One batch of 8 eggs, laid by a female in captivity, was laid very close together. After laying them, the female died and was dissected. The remaining eggs numbered 27, making a total of 35. Two more gravid females were dissected after they had died, and the eggs were counted. The total was 48 for one and 54 for the other.

Eggs were found from June 11 until August 24. The presence of large larvae indicated that some eggs had hatched as early as April. The average

number of eggs per 8-quart sample of soil was 83, the number decreasing toward the end of the season. The count included both hatched and unhatched eggs. On August 24 only one egg was found in a sample. The samples were taken from the upper 2 inches of soil, and each sample covered an area of approximately 1 square yard.

Eggs hatching in April, May, June, July, and August gave rise to larvae which pupated in March, April, May, June, July, and August of the following year. Adults emerged almost continuously from early March until August or September, the last swarm of adults occurring in early October. These were very small swarms and were made up predominantly of females which had probably emerged during September.

The larvae of the Bodega black gnat develop very slowly, requiring approximately 8 to 10 months to pass into the pupal stage. It is thought that there are four larval stages, although the second instar has not been obtained in captivity. Eggs have hatched into first-instar larvae; third-instar larvae have molted into fourth-instar larvae; and fourth-instar larvae have pupated; but first-instar larvae failed to molt into second-instar larvae in the laboratory. After hatching, the first-instar larvae measured 0.5 mm in length; they started feeding immediately after emerging from the eggs, which had been taken by the method described above. They lived in water for 3 weeks, feeding on organic material. They did not molt, and finally died. In consequence of this failure of first-instar larvae to molt in the laboratory, the second instar can only be inferred by size. Larvae which would be between the first and third instars by measurement have been taken by elutriation and by the heat-treatment method, but these died before molting.

Feeding larvae have often been observed with a binocular microscope. The mandibles alternately sweep into the mouth; or, at times, the head is moved in a scraping motion. Sand grains, as well as bits of organic matter, are scraped; mounted larvae show diatoms and fine detritus in the gut. Bacteria also are doubtless swallowed.

The larvae are found in thoroughly saturated soil, from 1 to 3 inches below the surface. In one rectangular block of soil, 12 x 12 inches in area, 166 larvae and 41 pupae were taken from samples 1 to 3 inches deep. Two larvae were taken in the 3- to 5-inch depth, but none in the 5- to 7-inch depth. The first larvae to pupate in the laboratory did so in March, shortly after the first larvae were found in the field.

The pupae wiggle actively to the surface of the sand before emergence, and stand erect on the tips of their abdomens, the rest of the body well out of the soil. They can be taken from the top of the soil in the field just before pupation if a careful scrutiny of the soil surface is made with a large reading glass. The pupal stage averaged 8 days in the laboratory. Pupae have been found in the field from early March to late August, August 24 being the latest date of recovery. They became less numerous in the sample as the season progressed.

BIOLOGY OF THE VALLEY BLACK GNAT

To discover the breeding areas, or larval habitats, of the valley black gnat, emergence traps, described in a previous paper (Smith, 1933), were placed in many localities in the field. Each trap covered 1 square yard of surface soil.

Although traps were placed in many types of environments, especially standing water and its environs, the gnats were found to emerge only from clay-adobe soil, particularly in sinkhole areas where water stood during the winter.

In order to build up a high concentration of larvae at one spot and thus facilitate their discovery later, two large cages were placed in the clay-adobe area near the emergence trap which had caught emerging adults. These cages were muslin-covered wooden frames 2 feet square and 3 feet tall. A smaller wire cage containing a live rat was placed inside each of the muslin cages. During the adult flight period, approximately one thousand female gnats were introduced into each cage. They fed readily upon the caged rats and, when fully engorged, crawled down the cracks in the adobe soil which formed the floor of the large cages.

At the end of the flight period, all surface dust and debris was carefully swept up from the bottom of the cages and examined microscopically for eggs, but none were found. Then the sweepings were moistened and observed from time to time for larvae but none appeared. This indicates that the eggs are not laid on the surface of the soil.

In March, nine months after the flight of the adults, the soil under the cages was carefully dug up and examined for larvae. The first larvae were found at a depth of 20 inches. Continued digging and searching yielded many more larvae and indicated that the adults had successfully matured their eggs on rat blood and had crawled into the cracks of the adobe soil to oviposit.

Larvae were found only in clay-adobe soil. The pH of the soil was 9.6 and the salt concentration 400 p.p.m. The majority of the soil particles were ultramicroscopic and exhibited Brownian movement in aqueous suspension. This soil has a marked capacity to swell when absorbing water; as it dries in the field it shrinks, forming large cracks an inch or more in width. The soil has a tendency to crack into hexagonal columns about 1 foot in diameter, but the hexagonal pattern is rarely achieved. At a depth of about 12 to 14 inches, the large surface cracks enter a system of narrower vertical and horizontal cracks which tend to divide the soil into cubes about 2 inches square. The crack system is a permanent feature of this soil, the same cracks opening each summer and closing again with the winter rains.

Attempts to Rear Larvae in the Laboratory. Many conditions were tested in the laboratory in attempts to rear larvae to adults. Various live foods, including small earthworms, nematodes, collembolans, and small chironomid larvae, were placed in moist dishes with gnat larvae but were not molested. A number of black-gnat larvae were confined in a small dish, but although they were frequently coiled around one another, they showed no tendency to cannibalism. The larvae readily ingested decayed plant material, but during a period of 30 to 60 days the fat body gradually disappeared and the larvae died, apparently of starvation. Larvae lived an equal time submerged in water containing organic matter. In water, they extruded two small disk-shaped pouches from the anus. It seems probable that the normal food of this species is organic detritus with its associated microorganisms—bacteria, fungi, and small nematodes. The failure to maintain fat and to pupate may be due to the relatively high oxygen content of laboratory media, in contrast to the very low oxygen content of clay soil at a depth of 2 feet.

Duration of the Larval Period. A group of 64 larvae, collected in April, were measured in length and were found to be classifiable into one of two well-defined size groups centering on 3.8 mm and 5.4 mm, as shown in figure 15. After the flight period of the adults had intervened in May and June, the larvae were almost as abundant in the soil as before. Measurements of 205 larvae collected in August showed two size groupings centering on 3.5 mm and 5.1 mm, as also shown in figure 15. These curves indicate: (1) that the minimum larval period is two years in duration, (2) that the summer is spent

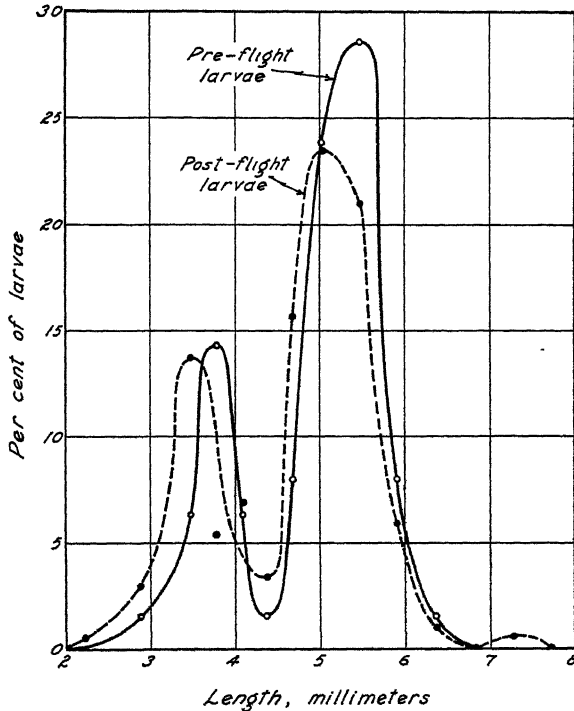


Fig. 15. Valley black gnat: lengths of larvae collected in the field just prior to and just after the flight of adults.

in complete aestivation, and (3) that many mature larvae do not transform into adults, but instead enter a diapause. Since the male and female adults are about the same size, the small larvae cannot logically be considered as one sex and the large larvae as the other. The only logical explanation is that the smaller larvae have fed through one winter and the larger larvae have fed through two or more winters. When larvae are found in the soil during the summer, they are in smooth, round burrows. Their bodies are usually bent double near the middle to form a closed U. They are practically motionless. When placed in water or wet soil they remain motionless for 3 days, then become active and crawl about. They seem to be in a state of immobile aestivation which can be broken by 3 days of contact with water. When larvae are brought into the laboratory in large unbroken clods of soil and no water is added, they remain in aestivation and are immobile.

Further evidence of diapause can be deduced from figure 15. The number of individuals in the first year of their development (80) is much smaller than those in their second year (187). This is the reverse of what would be expected if no diapause occurred. Without diapause the first-year larvae would be more numerous than second-year larvae, since the latter would have been reduced somewhat by natural mortality. If, however, some mature larvae go into a diapause, then large larvae would accumulate in the soil in greater numbers than small larvae. The smaller larvae are more difficult to find than the larger

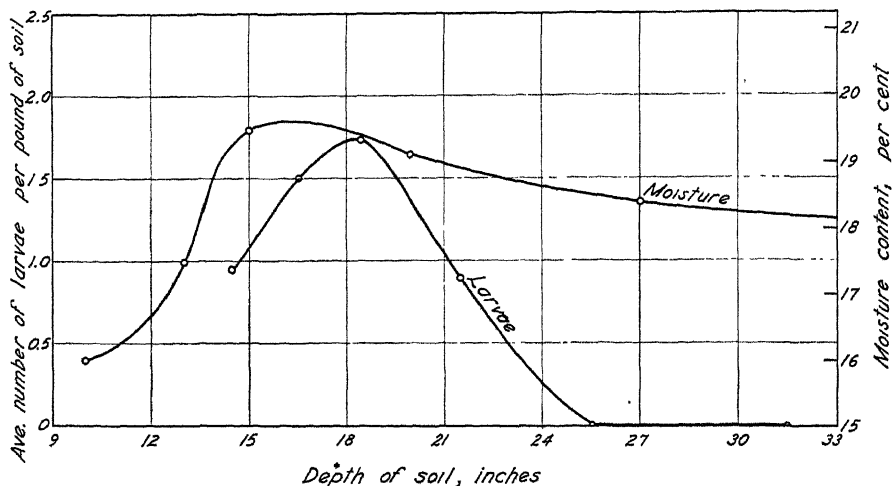


Fig. 16. Valley black gnat: depth of larvae in the soil in the field, and moisture content of the soil.

ones, but not sufficiently so to reverse an expected ratio of about 8 small to 6 large to the actual ratio of 8 small to 18 large. On the basis of these ratios, it is possible to assume that mature larvae had been going into a diapause for the past three years and that some larvae had lived in a mature condition in the soil for that length of time.

Moreover, if mature larvae did not diapause there would have been none in the soil after the flight period of the adults. Actually, in 1944, the ratio of small to large larvae in the preflight period was 1 to 2.31, and in the post-flight period was 1 to 2.20. This represents a loss of only 5 per cent of large larvae, which probably pupated and emerged. The small emergence in 1944 is confirmed by the opinions of long-time residents of the area, who stated that this gnat season was less annoying than usual.

As final evidence of diapause and aestivation, the following test was performed. On July 27, 1944, large clods of soil were collected containing mature larvae which had failed to emerge as adults 2 months earlier. These clods were trimmed to fit 1-gallon battery jars. No water was added. Glass covers were sealed on the battery jars with putty. On March 1, 1946, these jars were opened and live larvae found in the same number as at the start of the test. Their fat bodies were somewhat reduced as a result of long starvation. Since no

water was added it is probable that they did not come out of aestivation but remained dormant during the 19 months of this test.

Vertical Distribution of Larvae in the Soil. During August, 1944, a hole was dug in the breeding area and all larvae counted and their depth recorded. In this test 99 pounds of soil was examined minutely and 85 larvae were found. Their location in depth is shown graphically in figure 16. The moisture content of the soil at the time of digging was also measured and graphed. This graph shows that the optimum depth for larvae was 18 inches and that none were found below 25 inches. However, in other excavations, larvae have occasionally been found down to 38 inches.

Horizontal Distribution of Larvae. The larvae occur in sinkhole areas of clay-adobe soil. Water from winter rains collects in these depressions, and

TABLE 1
HORIZONTAL DISTRIBUTION OF LARVAE

Hole no.	Date sampled, 1944	Distance from bottom	Direction from bottom	Elevation above 0.0	Larvae per pound
		<i>yards</i>		<i>feet</i>	
1	July 27...	18	South	1 0	1.30
2	August 4	13	South	1.0	1.38
3	August 14	11	North	0 7	0 16
4	August 19	0	Center	0 0	0 05
5	August 28.	9	South	0 8	0 00
6	August 29...	22	South	1 1	0.48
7	September 1.	15	South	1.0	0.56

may stand there at a depth of from several inches to 2 feet during most of the winter. The relation of the distribution of larvae to these rain ponds was studied by digging 7 holes, each 3 feet square and 30 inches deep. An area 13 acres in extent was surveyed and contoured on a 2-inch vertical interval. The bottom of a depression 3 by 45 feet was designated 0.0 feet elevation. About 75 pounds of soil from each hole was carefully examined for larvae. The data are given in table 1.

During the winter of 1943-44, rain water accumulated to a depth of 3 or 4 inches and the center of the depression was under water about half of the winter. The fact that some larvae were found in the center of the depression, as well as the behavior of the larvae in water in the laboratory, indicates that they can tolerate submergence for a long period of time. On the other hand, the larvae at 1.0 foot elevation and above were probably never submerged at any time. The horizontal distribution of the larvae is believed to be a product of the requirements of the ovipositing female, as discussed later in this paper.

Pupation. Just prior to the flight period of the adults, usually early in May, some of the mature larvae leave their smooth-walled, cylindrical burrows and lie free between the permanent crack faces of closed cracks. As the soil dries out and the cracks begin to open, some environmental stimulus such as reduced humidity or increased oxygen content probably stimulates the larvae to pupate. Larvae oriented on crack faces occur at the usual depths, as mentioned earlier.

Extensive search has failed to yield pupae or pupal skins. It is believed that the pupal period is very short, probably about 5 days, in this species. (It has previously been shown that the pupal period of the Bodega black gnat is about 8 days.) Mature larvae have been kept in the laboratory under a variety of conditions in an attempt to induce pupation. These conditions included constant high and low temperatures, variable temperatures, wet and dry conditions and varying rates of desiccation; but none of the larvae pupated. It now appears that the conditions necessary for pupation are a constant temperature of about 68° F, a decreasing moisture content of the soil, and a low oxygen pressure.

Adults. The flight period of the adults is from 4 to 6 weeks, beginning usually in the middle of May. Variations in season from year to year may shift the flight period as much as 2 weeks one way or the other. The emergence from any one spot probably does not extend over more than 3 weeks, as shown by the following catch from a single trap which stood continuously on the same square yard of soil:

Date 1945	Males	Females	Rainfall, inches
May 15	0	0	0.11
May 20	0	0	0.00
May 21	3	0	0.00
May 22	6	3	0.00
May 24	7	8	0.00
May 26	10	9	0.00
May 28	13	14	0.00
May 29	12	15	0.00
May 30	1	2	0.09
May 31	1	0	0.05
June 1	2	1	0.01
June 2	21	39	0.00
June 3	10	8	0.00
June 4	5	1	0.00
June 5	0	0	0.00
June 7	3	3	0.00
June 8	0	2	0.00
June 9	1	1	0.00
June 10	0	1	0.00
June 11	0	0	0.00

The rainfall and cold weather of May 30 to June 1 retarded the emergence of the gnats. Ordinarily, the great majority of the gnats would probably have emerged within a 10-day period. The sex ratio of the emerging gnats, averaged for all traps, was 1 male to 1.14 females.

The gnats crawl out of the cracks in the soil about 8 o'clock in the morning and climb up on vegetation to sun themselves until about 10 o'clock, when the temperature reaches the point at which they fly. Prior to this time many gnats can be swept from the vegetation with an insect net, but none are flying. Dancing swarms of males have never been found in the Sacramento Valley, but large swarms of several hundred each are common in the Santa Clara Valley. Females have been observed to bite under natural conditions as late as 8 p.m. on warm still evenings.

Feeding, and Symptoms on Man. Unfed gnats are voracious and fearless; they cannot be frightened away from the host. They usually run about for a few seconds on the host in an exploratory fashion. They then settle down with the legs well spread and braced. The mouth parts seem to saw for about $1\frac{1}{2}$ minutes, then the abdomen quickly fills with blood, becoming considerably distended. The host feels no sensation during the first 30 to 60 seconds of the bite; after that a slight tingling is noticeable. Defecation occurs as the gnat's abdomen distends; a clear colorless fluid is extruded. Sometimes this droplet is tinged with the newly ingested blood. After feeding, the gnat is very easily frightened away, or may leave voluntarily; but if not disturbed, it may remain at the site of the bite, cleaning its body. A pin-point hemorrhage remains under the skin of the host at the locus of the bite, showing that one or more capillaries have been cut. The feeding process of the Bodega black gnat is similar.

In about 10 to 15 minutes there appears a raised area, hard and white, about $\frac{1}{2}$ cm in diameter, still characterized by the minute red dot in the center. Itching is now pronounced. One and one-half hours after the bite, the hard white area is partially reduced and has become inflamed. A diffused, red blotchy area 1 to 2 inches in diameter appears around the central swelling. Three hours after the bite, the diffused red area disappears, leaving the central $\frac{1}{2}$ -cm area red and resembling a flea bite. Itching has disappeared. Six hours after the bite is inflicted, all swelling and most of the inflammation has disappeared, and itching has stopped. At this stage, it is difficult to locate the bites. Eighteen hours after the bite, the spot is marked by a hard inflamed conical swelling about $\frac{1}{4}$ cm in diameter. The hemorrhagic center has faded but can still be seen. Itching has returned and is moderate. On many persons a small clear blister forms at the locus of the bite on the second day and disappears a day or two later. Itching is intense for about 5 days, especially early in the morning.

The time required to complete feeding is usually 2 minutes, but occasionally takes as long as 4 minutes. A gnat which has once fully engorged will not feed again, but will drink water eagerly.

Gnats have been observed feeding on chickens, turkeys, dogs, cats, and rats. Townsend (1893) reports them feeding on horses. It is probable that they will feed on any warm-blooded animal. On such hosts they burrow quickly into the fur or feathers as soon as they alight.

One female, newly emerged from the soil, fed on a male of her species in captivity, and later, when dissected, was found to contain mature eggs. In the absence of warm-blooded hosts, this species might maintain itself by this means.

Longevity. Without food, the adults live only 6 to 8 hours. Water does not prolong their lives. Females which were fed on human blood lived a maximum of 5 and an average of 4 days. Their death was believed to be due to egg pressure and ruptured oviducts: when dissected, the abdomen was found to be full of eggs and often the oviducts were ruptured.

Eggs. Various attempts to simulate field conditions and secure egg deposition in the laboratory failed. In these tests human blood and rat blood were used as food. Gravid females were dissected and the mature eggs were found

times. Males form large swarms, dancing in the lee of windbreaks. Eggs are laid on the surface of damp sand where the salt concentration is about 640 p.p.m. The larval stage lasts for 8 to 10 months. Pupation occurs in the sand; pupae wriggle to the surface and stand vertically before the adult emerges. The pupal period is 8 days. Adult females captured in the field lived a maximum of 11 days, with blood meals. Males do not feed; they lived a maximum of 4 days after capture in the field.

Adults of the valley black gnat occur for 4 to 6 weeks, beginning usually in the middle of May. Females feed only once; males do not feed. Unfed gnats live only 6 hours in captivity; with a blood meal, females live a maximum of 5 days. The larvae occur in clay-adobe soils at a depth of 15 to 30 inches. Egress and entrance is dependent upon the drying and cracking of the soil. The larval period is at least two years in length. Larvae spend the summers in immobile aestivation. If the soil does not crack on schedule, the mature larvae enter a diapause. Some evidence is given to indicate that larvae may diapause for at least three years. Larvae are found in summer in soil with a moisture content of 17 to 20 per cent, a salt concentration of 400 p.p.m., a pH of 9.6, and a temperature of 65° to 68° F. Methods of identifying breeding grounds consist of trapping adults as they emerge from the soil, and washing larvae from the soil.

Both species feed viciously on man, domestic animals, and birds. The bite usually produces a transient inflammatory swelling and often an intense itching which may continue for about 1 week.

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**CHARACTERS, DISTRIBUTION, AND FOOD PLANTS OF
LEAFHOPPER SPECIES IN THAMNOTETTIX GROUP**

DWIGHT M. DELONG and HENRY H. P. SEVERIN

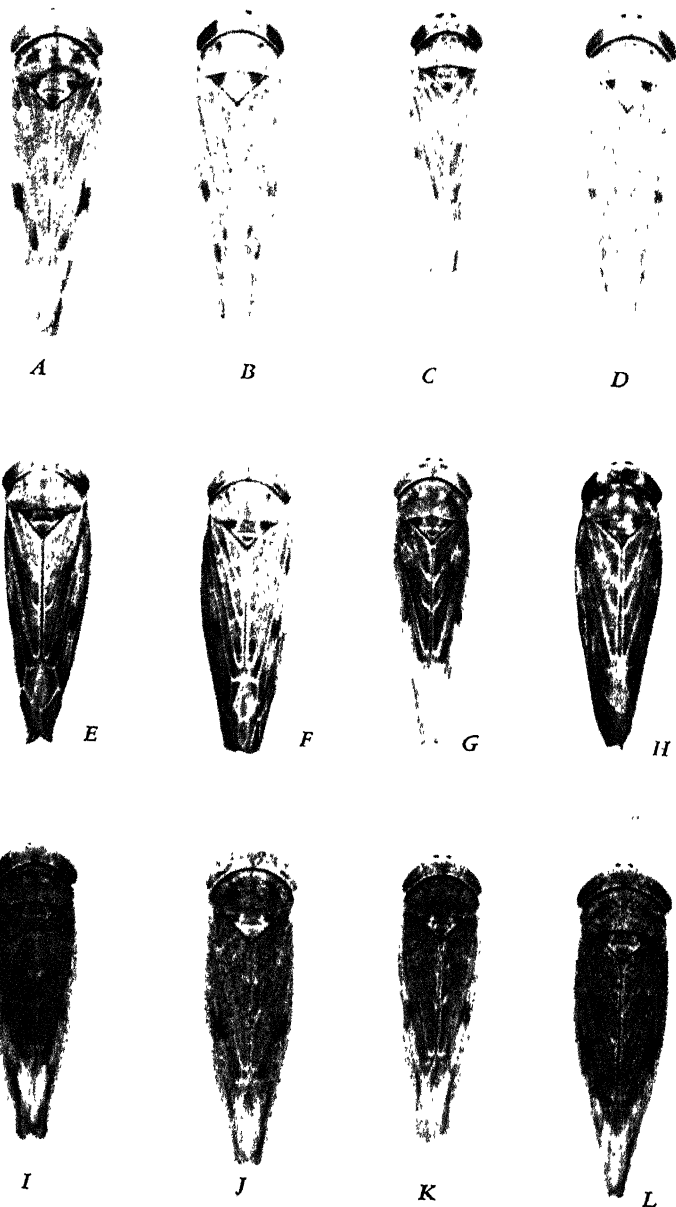


Plate 1.—Color variations of *Idiodonus heidemanni* (Ball), a leafhopper vector of California aster-yellows virus: A, B, gray females with no spot and one black spot on front of head, respectively; C, gray male, and D, gray female, with two round black spots between ocelli; E, F, brown females with no spot and one spot on front of head respectively; G, brown male, and H, brown female, with two round black spots between ocelli; I, male, and J, female, with no spots on front of head and blood-red dots on head, thorax, and wings; K, male; L, female with two round black spots between ocelli and blood-red dots on head, thorax, and wings respectively.

CHARACTERS, DISTRIBUTION, AND FOOD PLANTS OF LEAFHOPPER SPECIES IN THAMNOTETTIX GROUP¹

DWIGHT M. DELONG² and HENRY H. P. SEVERIN³

INTRODUCTION

SOME years ago three species (Severin, 1929, 1934)⁴ and a biological race (Severin, 1940) of one of these leafhopper species were reported to transmit the California aster-yellows virus. Recently, DeLong and Severin (1945, 1946, 1947a, 1947b) recorded thirteen additional leafhopper vectors of the virus. The present paper deals with the characters, distribution, and food plants of eight more leafhopper vectors, two of which have been previously mentioned in the literature (Severin, 1934). In a companion paper Severin (1948) discusses the transmission of the virus by these eight leafhopper species.

The genus *Thamnotettix* was erected by Zetterstedt (1840) to include European species, and *Cicada prasina* Fallen was designated as the type. The early American workers placed a large number of American species in this genus as they were described. In recent years several new genera have been described to include certain groups of closely related American species formerly in the genus *Thamnotettix*. The species treated in the present paper have been placed in three genera described by Ball (1936). These are *Idiodonus*, *Colladonus*, and *Friscanonus*. There is little doubt that these species in the three genera are closely related; they may belong to a single genus. The color patterns will usually distinguish them, but the genital structures are similar in both the males and the females of the species concerned. The females usually bear a median sunken spatulate process on the last ventral segment which varies in width, length, and the degree of production beyond the posterior margin in different species. The males may be distinguished by the shape of the style and the length and position of the spine on each side of the caudal margin of the pygofer.

IDIODONUS HEIDEMANNI (BALL)

Idiodonus heidemanni (Ball) is blunt-headed and has a general color of grayish green, sprinkled with minute red spots. It is 4 mm long.

The vertex (fig. 1, A) is broad, bluntly produced, and about twice as wide at the base between the eyes as the median length.

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⁴ See "Literature Cited" for citations, referred to in the text by author and date.

The vertex and face are pale yellow; the face has several fuscous arcs. The pronotum is pale, dull green; the anterior portion is paler. The scutellum is yellow with an orange spot in each basal angle. The elytra are milky white and subhyaline, with a greenish tint. The entire upper surface and the face are closely dotted with minute, reddish, pepperlike spots.

The last ventral segment of the female (fig. 1, *B*) is roundedly produced and bluntly rounded at the apex. The male plates are rather broad at the base,

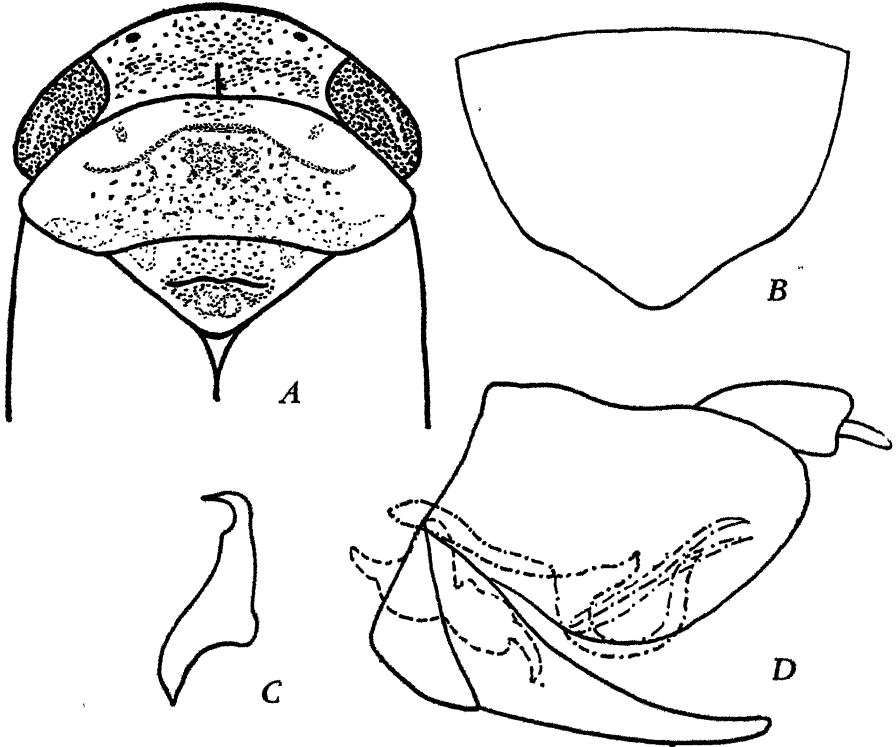


Fig. 1. *Idiodonus heidemanni* (Ball): *A*, dorsal view of head, pronotum, and scutellum; *B*, female last ventral segment; *C*, ventral view of male style; *D*, lateral view of male genitalia.

and roundedly narrowed to form slender, elongated, tapering apices. The male style (fig. 1, *C*) is rather broad at the base and rather short, narrowed to a pointed apex, which is curved outwardly and directed laterally. The aedeagus (fig. 1, *D*) is sicklelike in lateral view, with a pair of long slender processes extending dorsally and caudally from the base of the sickle beyond its apex. The pygofer is rounded without a spine at its apex.

Breeding Experiments. Ball (1900) described *Idiodonus heidemanni* (Ball) (= *Thamnotettix heidemanni* Ball) collected from Cerro Summit and Alder, Colorado, both high mountain points. Ball (1911) also described *I. schwartzi* (Ball) (= *T. schwartzi* Ball) from a pair taken at Dewey, Utah, by J. R. Horton, and from one female taken at Ashford, Arizona, by Barber and Schwartz; the latter he received from the United States National Museum.

Ball (1900) described the color of *Idiodonus heidemanni* as grayish green sprinkled with blood-red dots; the detailed color description is as follows:

The vertex and face pale yellow, sutures and about five short arcs on the front fuscous, pronotum pale olive, the anterior margin lighter, scutellum yellow, an orange spot inside each basal angle. Elytra milky subhyaline with a greenish cast, the black tergum showing through. Whole upper surface and face minutely dotted with blood red.

Ball (1911) described *Idiodonus schwartzi* as smoky cinereous, with two round black spots on the front of the head, and two angled ones on the scutellum; his description is as follows:

The vertex pale yellow, slightly washed with orange, the ocelli red, a pair of round black spots between them equidistant from the ocelli and each other. Face pale yellow, the sutures dark, a few short smoky arcs on lower part of front. Pronotum cinereous. Scutellum yellow, a triangular black spot just within each basal angle. Elytra cinereous, the costal margin subhyaline, a narrow smoky stripe at apex. Veins of clavus and claval suture pale, veins on corium and a line along the claval suture smoky, emphasized on a line which follows the outer sector omitting its outer branch, and ends in the margin of the third apical cell.

In breeding experiments by the junior author, pairs of recently molted adults were mated, the males fitting the description of *Idiodonus schwartzi*, the females similar except that some had no spots, some 1 black spot, and some the typical 2 spots between the ocelli. Each pair without exception had some offspring with no spots, some with 1 spot, and some with 2 spots, as shown in plate 1, *A* to *H*. Males and females with blood-red dots on the body (plate 1, *I, J, K, L*), and otherwise fitting the description of *I. heidemanni*, were mated. Some of the offspring of each pair failed to show the red spots. Females with acute and rounded heads were mated, each with males of the same type. The offspring of pairs with acute heads had both acute and rounded heads; and the offspring of those with rounded heads likewise had both acute and rounded heads. During the winter most of the adults are brown in color (plate 1, *E, F, G, H*) and during the summer gray forms (plate 1, *A, B, C, D*) predominate. This is true both under natural conditions and under greenhouse conditions. This breeding evidence indicates that we are dealing with color variations of a single species. The priority name which must be given to this species is *Idiodonus heidemanni*.

Geographical Range. The detailed occurrence of *Idiodonus heidemanni* is not known, but it is recorded for California and Colorado (Ball, 1900). It probably occurs in other states of the northwestern United States.

Distribution and Food Plants in California. The localities in which *Idiodonus heidemanni* was collected and its food plants are as follows:

Los Angeles County: This leafhopper species was commonly taken during the summer of 1919 on Australian saltbush, *Atriplex semibaccata*, near Compton by H. H. P. Severin.

Santa Barbara County: Near Santa Maria and Lompoc, on July 31, 1942, adults were collected in sugar-beet fields by N. W. Frazier.

Napa County: On October 4, 1945, 1 female was captured on an unknown host plant at Larkmead by H. H. P. Severin.

Marin County: Adults were abundant on alkali heath, *Frankenia grandifolia*, October 10, 1946, and July 27, 1947, near San Rafael.

IDIODONUS KIRKALDYI (BALL)

Idiodonus kirkaldyi (Ball) is a pale green to yellowish species with black spots on the vertex, somewhat resembling *Colladonus geminatus* in appearance. It is 3.5 to 4.0 mm long.

The vertex (fig. 2, A) is produced and bluntly rounded at the apex. The length at the middle is about the same as the basal width between the eyes.

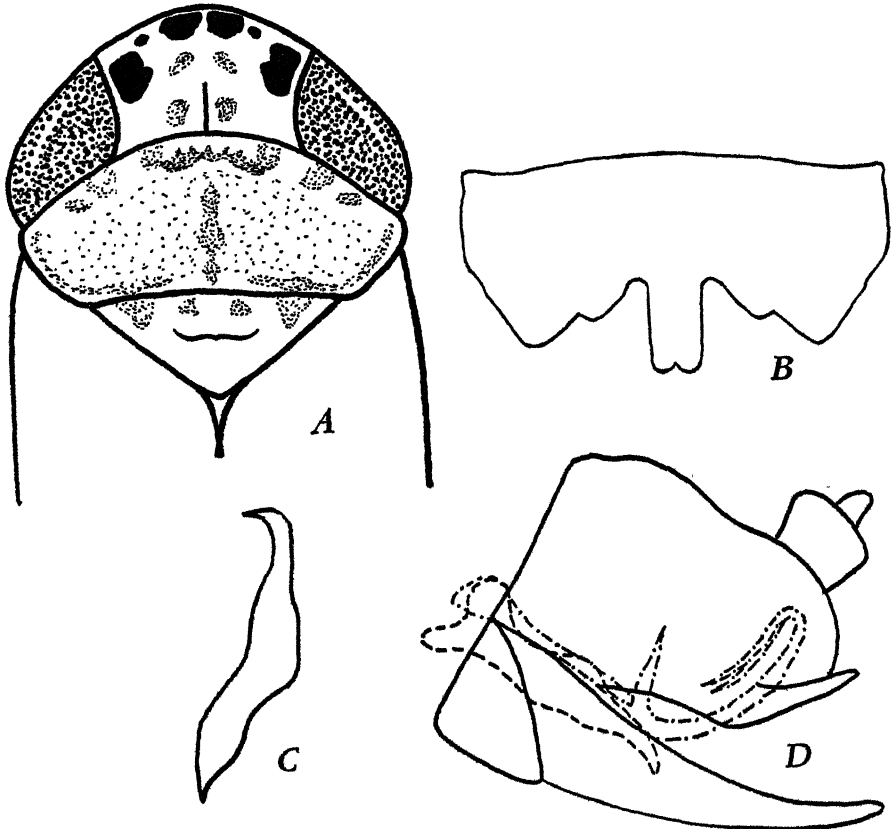


Fig. 2. *Idiodonus kirkaldyi* (Ball): A, dorsal view of head, pronotum, and scutellum; B, female last ventral segment; C, ventral view of male style; D, lateral view of male genitalia.

The color of the vertex is creamy yellow with a pair of large, round, black proximal spots at the apex, and a large, round, black spot just back of each ocellus next the eye. A pair of rather large brownish spots are on the base of the vertex; each spot is about equidistant between the eye and the median line. The pronotum is dull yellow to pale brownish, with irregular dark mottling along the anterior margin and on the disk. The scutellum is creamy yellow, with triangular brown spots in the basal angles and a pair of small, round, black spots between them, just back of the anterior margin. The elytra are pale brownish; the costal portion is subhyaline, and the nervures are pale,

usually margined with brown. The elytra appear striped because of a narrow, dark, smoky stripe on the claval area and a wider stripe just inside the outer sector of the corium; the wide stripe extends across the first and second ante-apicals and the second apical cell.

The last ventral segment of the female (fig. 2, *B*) is angularly excavated from the prominent lateral angles to the base of a median spatulate process; this process is almost parallel-margined, slightly notched at the apex, and produced beyond the posterior margin of the segment. The male plates are rather broad at the base, then roundedly narrowed to form long, attenuated, tapering apices. The style (fig. 2, *C*) is elongate, only slightly narrowed on the apical fourth, with the apex bent laterally and sharp-pointed. The aedeagus (fig. 2, *D*) is slender, almost parallel-margined, and curves dorsally almost to the anal tube; there it recurves and is divided into a pair of slender processes which extend ventrally. The pygofer bears a spine, which arises on the ventral apical portion and only slightly exceeds the rounded margin of the pygofer.

Geographical Range. *Idiodonus kirkaldyi* apparently occurs only in California.

Distribution and Food Plants in California. High populations of *Idiodonus kirkaldyi* were collected during the summer and autumn on California sage-brush, *Artemisia californica*, in San Mateo County.

GEMINATE LEAFHOPPER, *COLLADONUS* *GEMINATUS* (VAN DUZEE)

The geminate leafhopper, *Colladonus geminatus* (Van Duzee) is a small, blunt-headed, greenish species with black markings similar to those of *Idiodonus kirkaldyi*. It is 4.5 mm long.

The vertex (fig. 3, *A*) is bluntly and roundedly produced, and almost twice as wide between the eyes at the base as the median length. The ocelli are about one third the distance from the eyes to the apex.

The vertex is yellow in color, with a pair of large triangular black spots on the margin at the apex. An elongate somewhat quadrate black spot is just back of each ocellus. A small brownish spot is just posterior to the inner margin of each of the latter black spots. The pronotum is yellowish anteriorly with a few brown spots along the anterior margin, and the disk is dull greenish brown. The scutellum is dull yellowish, with a median impressed brown line, a pair of round brown spots just in front of it, and a brownish triangular spot about half way from the basal angle to the median line on each side along the anterior margin. The elytra are brown to greenish subhyaline; the nervures are pale except on the apical portion, where they are brown.

The female last ventral segment (fig. 3, *B*) is rounded to the posterior margin, which is somewhat sinuate and distinctly notched either side of a short, rather broad, median spatulate process. This process is produced beyond the posterior margin of the segment and slightly notched at middle. The male plates are broad at the base, then concavely narrowed to rather long acutely pointed apices. The style (fig. 3, *C*) is elongate and rather narrow, more strongly narrowed on the apical sixth. The apex is blunt with a slight toothlike projection on the outer apical margin. The aedeagus (fig. 3, *D*) is slender, almost parallel-margined, with a pair of terminal processes which extend

ventrally from the recurved dorsal portion. The pygofer bears a prominent spine on each side at about the middle of the apical portion.

Geographical Range. The geminate leafhopper is quite common and widely distributed in the northwestern United States and in western Canada. It is known to occur in Alaska, British Columbia, Washington, Oregon, California, Colorado, Utah, Wyoming, Montana, and Idaho.

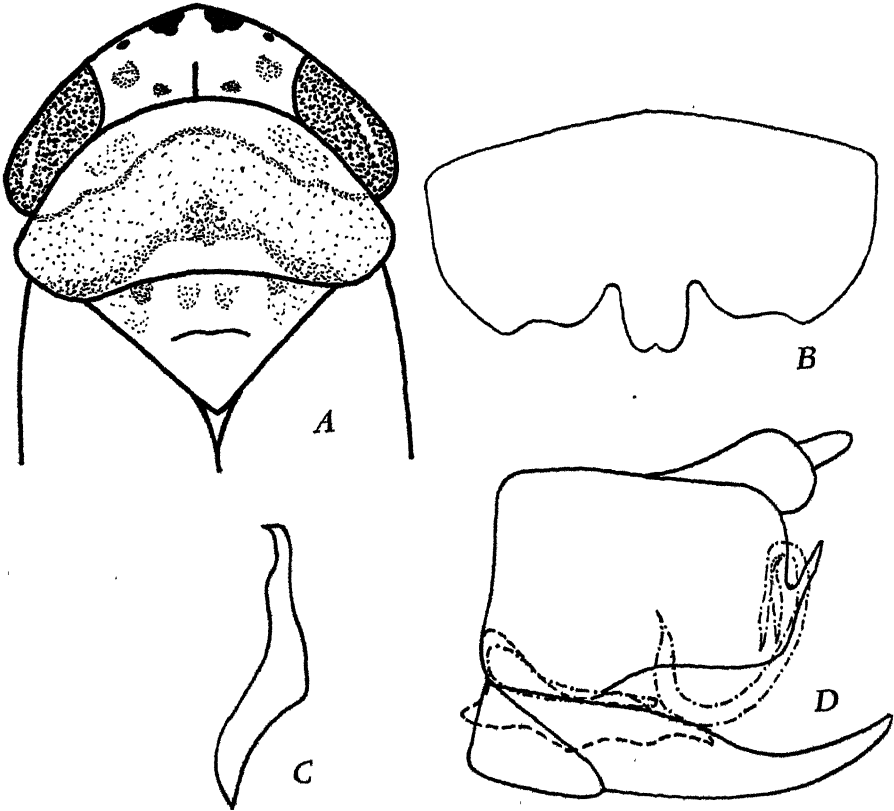


Fig. 3. *Colladonus geminatus* (Van Duzee): A, dorsal view of head, pronotum, and scutellum; B, female last ventral segment; C, ventral view of male style; D, lateral view of male genitalia.

Distribution and Food Plants in California. The geminate leafhopper is widely distributed in California and has been taken in vegetable fields and on ornamental flowering plants. It has commonly been collected on carrots in the Sacramento and Salinas valleys but rarely on celery, and often on asters in the Salinas Valley (Severin, 1934). Nymphs and adults are abundant on delphiniums and are an efficient vector of the virus to this host plant (Severin, 1942).

MOUNTAIN LEAFHOPPER, *COLLADONUS* *MONTANUS* (VAN DUZEE)

The mountain leafhopper, *Colladonus montanus* (Van Duzee) is a blunt-headed species with a tiny yellow saddle on the commissural line of a pair of black elytra. It is 4.5 mm long.

The vertex (fig. 4, *A*) is bluntly angled and almost twice as wide between the eyes at the base as the median length. The color of the vertex is pale yellow to white, with a darker band on the basal portion between the eyes. The pronotum is pale yellowish except for a darker band on the anterior portion

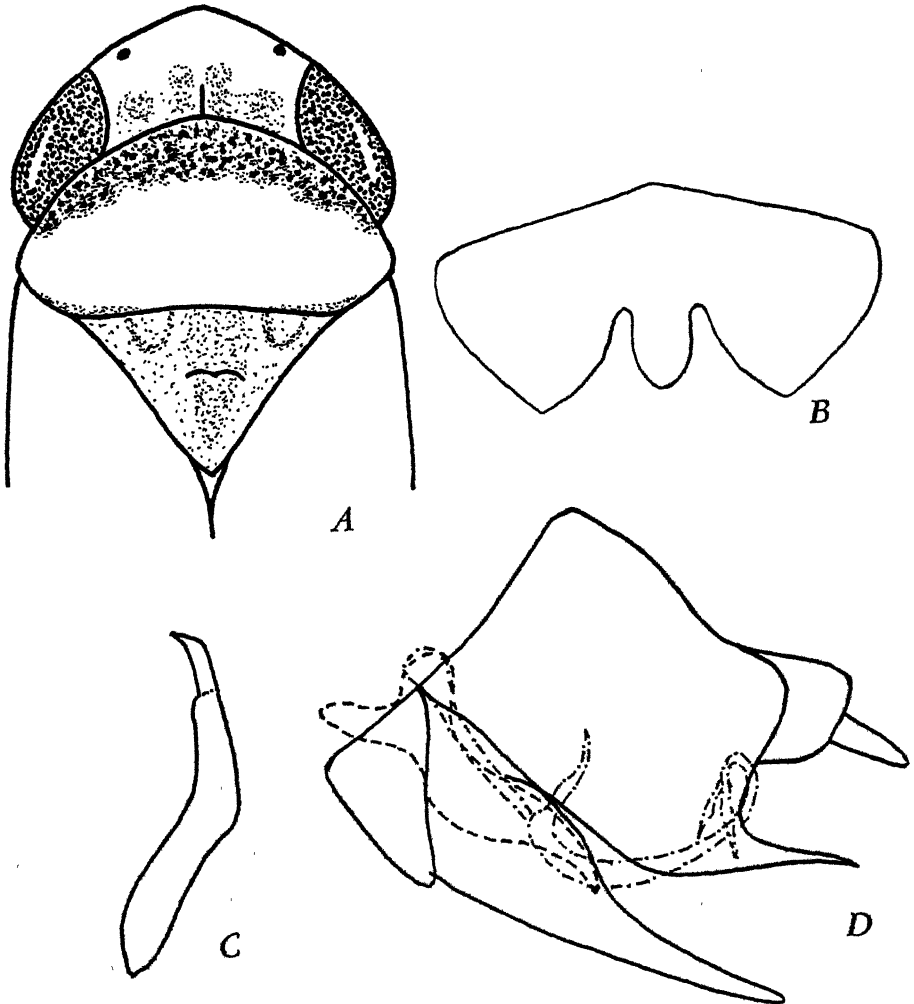


Fig. 4. *Colladonus montanus* (Van Duzee): *A*, dorsal view of head, pronotum, and scutellum; *B*, female last ventral segment; *C*, ventral view of male style; *D*, lateral view of male genitalia.

between the eyes. The scutellum is dark brown to black. The elytra are dark brown or black; the costal half of the corium as far as the apical cell is hyaline with a tiny yellow spot on the corium of the clavus. The face is pale with darker arcs.

The posterior margin of the last ventral segment of the female (fig. 4, *B*) bears a spatulate process at the middle which is produced to the length of the posterior margin and is separated from the broad produced lobes on either

side by a deep U-shaped excavation. The male plates are elongate triangular, with long tapering apices. The male style (fig. 4, *C*) is elongate, only slightly narrowed at the apex. The outer apical margin is pointed. The aedeagus (fig. 4, *D*) extends dorsally, then curves ventrally, forming a pair of slender processes. The pygofer bears a long sharp apical spine on each side, which is directed caudally from the lower portion of the pygofer. This is formed by a deep concave excavation on the dorsal apical portion of the pygofer just beneath the anal tube.

Geographical Range. The mountain leafhopper is widely distributed in the Pacific Northwest: it is known to occur in California, Oregon, Washington, Montana, Wyoming, Idaho, Colorado, and Utah in the United States, and in British Columbia in Canada.

Distribution and Food Plants in California. The mountain leafhopper is generally distributed in California and has been taken on many different vegetables. During the summer and autumn of 1931 this leafhopper was very abundant in celery fields near Sacramento. Celery was so generally infected with the California aster yellows that it was plowed under (Severin, 1934). Adults captured in the celery and in delphinium fields transmitted the virus to healthy celery; it was thus demonstrated that the insect is a vector of the virus under natural conditions (Severin, 1934, 1942). This leafhopper is one of the most important vectors of the virus to delphinium and breeds on this host plant under natural conditions. A list of economic plants and weeds which serve as food plants of this leafhopper has been published in a previous paper (Severin, 1934).

COLLADONUS COMMISSUS (VAN DUZEE)

Colladonus commissus (Van Duzee) is yellow tinted with orange; it has a bluntly produced head and is 5 to 6 mm long.

The vertex (fig. 5, *A*) is bluntly angled, more than one third longer at the middle than the basal width between the eyes.

The color is straw yellow, tinted with orange, and there are no distinct markings. The pronotum is yellowish, often with a broad triangular spot on the posterior half. The scutellum is yellowish with darker basal angles. The elytra are pale brownish subhyaline, with paler veins except those on the apical portion. The face is pale with faint arcs.

The female last ventral segment (fig. 5, *B*) is strongly produced to form lateral angles, between which the posterior margin is deeply and angularly notched either side of a median rather broad spatulate process, which is not produced to the posterior margin of the segment. The male plates are elongate and triangular. The style (fig. 5, *C*) is elongate and rather narrow, the apical fourth is more narrowed, and the apex bears on the outer margin a rather long-pointed tooth, which extends laterally. The aedeagus (fig. 5, *D*) is rather narrow and curves dorsally, extending almost to the anal tube, where it re-curves and divides into two slender terminal processes, which extend ventrally. The pygofer spine, which is on the middle of the caudal portion, is conspicuous.

Geographical Range. The known records indicate that *Colladonus commissus* has been taken only in California.

Distribution and Food Plants in California. The locality in which *Colladonus commissus* was collected, the food plants on which it was found, and the numbers collected are as follows:

San Mateo County: At Montara, on September 28, 1941, 2 females were collected on California blackberry, *Rubus vitifolius*. On July 31, 1942, 3 males and 9 females were taken on bush lupine, *Lupinus arboreus*, growing in a

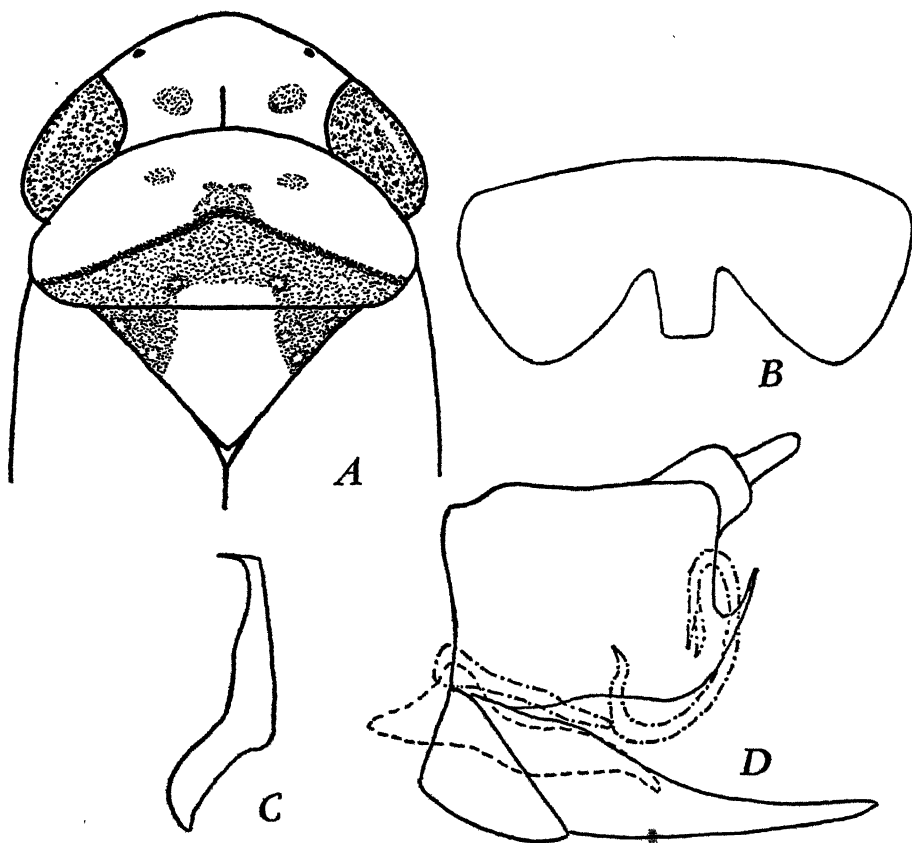


Fig. 5. *Colladonus commissus* (Van Duzee): A, dorsal view of head, pronotum, and scutellum; B, female last ventral segment; C, ventral view of male style; D, lateral view of male genitalia.

canyon near Montara. This leafhopper was commonly collected on monkey-flower, *Diplacus aurantiacus*, during the spring, summer, and autumn each year from 1943 to 1945. It was rarely taken on bracken, *Pteridium aquilinum* var. *lanuginosum*, near Montara. On July 25, 1945, 1 female was captured on Aleppo pine, *Pinus halepensis*, growing in Sharp Park.

Alameda County: At Berkeley, on August 28, 1942, 1 female was swept from Japanese or Boston ivy, *Parthenocissus tricuspidata*.

Sonoma County: On June 4, 1943, a few adults were collected on an unknown host plant by N. W. Frazier.

COLLADONUS FLAVOCAPITATUS (VAN DUZEE)

Colladonus flavocapitatus (Van Duzee) has a sharply pointed head, which is yellow without markings; the elytra are brown. It is 5.0 to 5.5 mm long.

The vertex (fig. 6, A) is produced and angled. The apex is pointed. The median length is more than one half the basal width between the eyes.

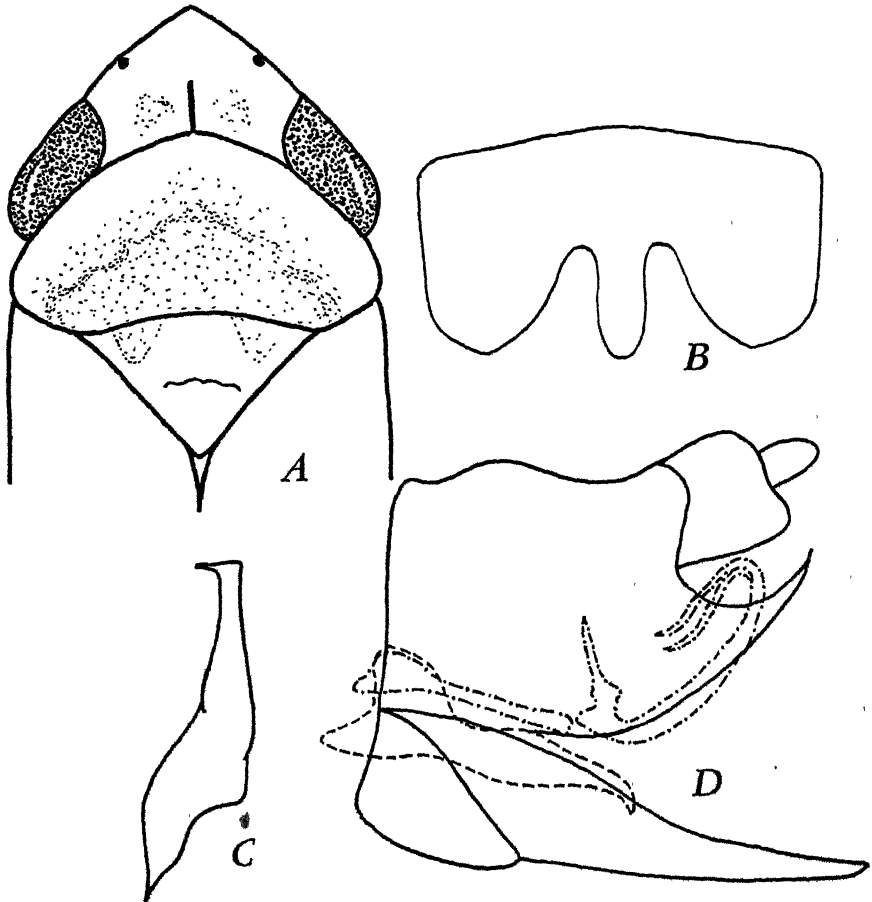


Fig. 6. *Colladonus flavocapitatus* (Van Duzee): A, dorsal view of head, pronotum, and scutellum; B, female last ventral segment; C, ventral view of male style; D, lateral view of male genitalia.

The vertex of the male is yellowish, tinted with brown. In the female it is often darker. The pronotum is olive brown, the anterior margin is paler. The scutellum is reddish brown. The elytra are some shade of brown or greenish brown. The face is pale yellow.

The female last ventral segment (fig. 6, B) is produced and rather broadly rounded on the outer margins. The posterior margin is deeply excavated either side of a median spatulate process; this process is rounded at the apex and pro-

duced to the length of the posterior margin of the segment. The male plates are elongated triangular, rather broad at the base and rounded, tapering to acute apices. The style (fig. 6, *C*) is elongate, gradually tapered to the apex; the apex is blunt and bears on the outer margin a long toothlike projection, which is directed laterally. The aedeagus (fig. 6, *D*) is directed dorsally and recurved, with a pair of slender processes which extend ventrally. The pygofer bears a long curved apical spine on each side, which is formed by a deep excavation just below the anal tube.

Geographical Range. *Colladonus flavocapitatus* is widely distributed in the western portion of the North American continent: it occurs from Alaska through British Columbia and Alberta to Washington, Oregon, California, Idaho, and Colorado.

Distribution and Food Plants in California. As determined by N. W. Frazier and J. H. Freitag, the localities and food plants of populations collected are as follows:

Tulare County: Adults were commonly swept from wild gooseberry, *Ribes* sp., growing in General Grant Park, on September 3, 1940; September 3, 1942; September 11, 1943; and September 15, 1944; by N. W. Frazier and J. H. Freitag. Adults were taken on *Ribes cereum* and on *R. roezlii* on August 14, 1947, by H. H. P. Severin.

Sonoma County: On September 11, 1943, 1 male was collected, but the host plant is unknown.

FRISCANANUS INTRICATUS (BALL)

Friscananus intricatus (Ball) has a pointed head and the vertex is without distinct markings. The length is 5 mm.

The vertex (fig. 7, *A*) is pointed at the apex, slightly longer at the middle than the basal width between the eyes. The anterior margin is rounded to the front except at the apex. The elytra contain several irregular reticulate veinlets on the clavus and in the antepical cells.

The color of the vertex is pale, washed with brown. There is usually a pale band before the eyes. The face is pale with fuscous arcs. The pronotum is usually dark brown with an anterior bow-shaped pale line. The elytra are dark brown with paler nervures, and the irregular reticulations are usually milky white. There is usually an oblique, subhyaline, light area beyond the middle of the costa.

The female last ventral segment (fig. 7, *B*) is concave on the posterior margin, with an angular emargination either side of a median spatulate process, which is rather broad and considerably exceeds the posterior margin in length. The male plates are long and taper to attenuated apices. The style (fig. 7, *C*) is elongate and rather narrow. It is more narrowed on the apical fourth. The apex is bent sharply so as to form a long sharp toothlike projection on the outer margin. The aedeagus (fig. 7, *D*) is narrow and curved dorsally, then recurved ventrally at the anal tube; there it divides into a pair of long slender processes, which are directed ventrally. The pygofer bears a very short pointed spine at about the middle of the caudal margin.

Geographical Range. The records of *Friscananus intricatus* indicate that it occurs only in California.

Distribution and Food Plants in California. Adult *Friscananus intricatus* were taken in small numbers during the spring, summer, and autumn of 1941 to 1945 on monkey-flower, *Diplacus aurantiacus*, in San Mateo County. At Montara, July 25, 1945, it was occasionally collected on bracken, *Pteridium aquilinum* var. *lanuginosum*.

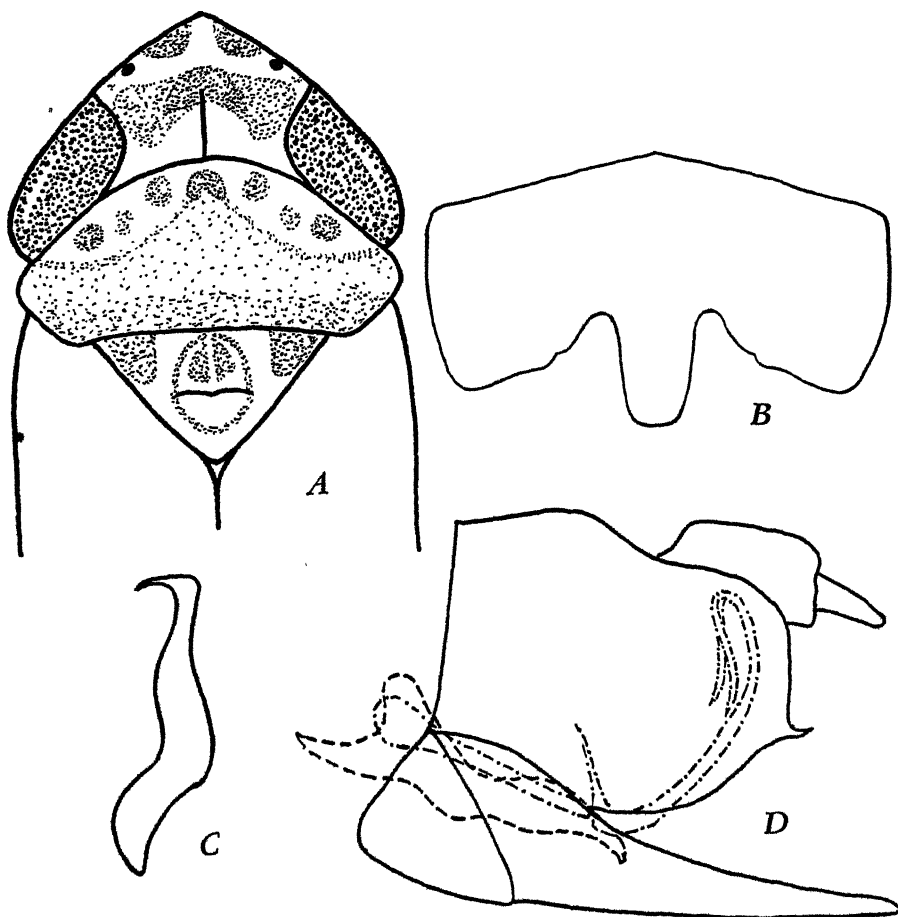


Fig. 7. *Friscananus intricatus* (Ball): A, dorsal view of head, pronotum, and scutellum; B, female last ventral segment; C, ventral view of male style; D, lateral view of male genitalia.

FRISCANANUS RUPINATUS (BALL)

Friscananus rupinatus (Ball) resembles *F. intricatus* in having a produced, pointed vertex. In addition it has a bisected black spot at the apex. It is 5 mm long.

The vertex (fig. 8, A) is obtusely angled, the apex is rounded, and it is as wide between the eyes at the base as the median length. The elytra do not contain extra reticulate veinlets.

The vertex is orange yellow with a large semicircular black spot on the apex, bisected by the narrow, pale, median line. The pronotum is greenish to reddish brown; the anterior margin is pale. The scutellum is pale yellow; the basal angles are greenish brown. The elytra are greenish subhyaline, washed with brown. The venation is usually inconspicuous. The apices of the claval veins are white, and the veinlets surrounding the apical cells are rusty brown.

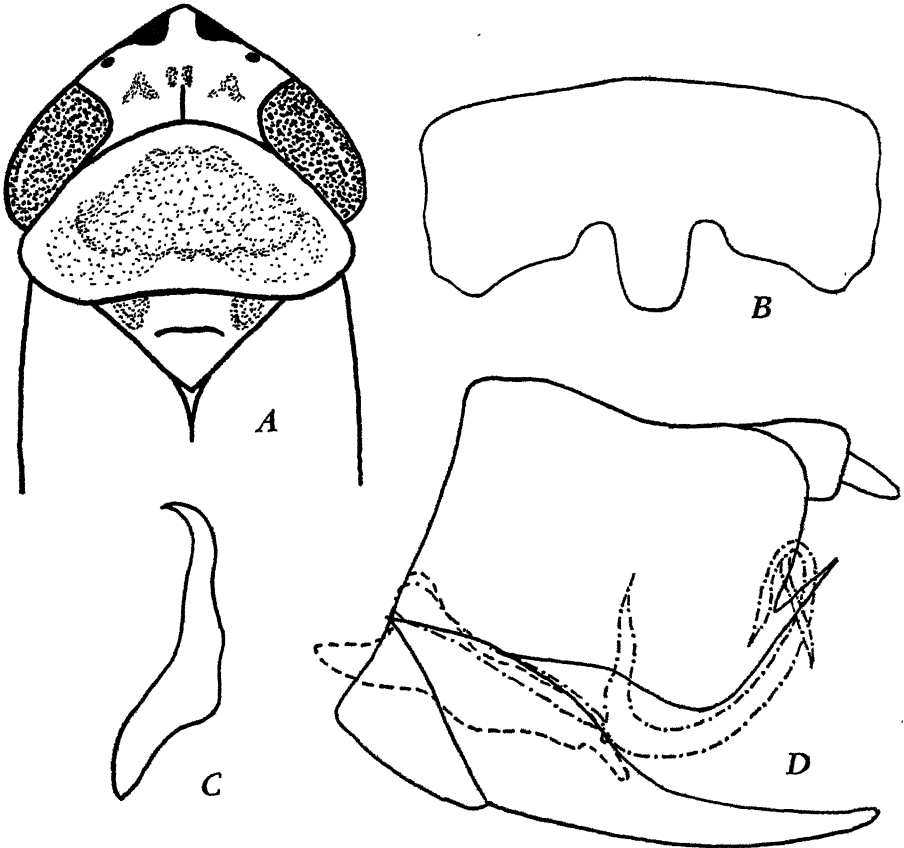


Fig. 8. *Friscoananus rupinatus* (Ball): A, dorsal view of head, pronotum, and scutellum; B, female last ventral segment; C, ventral view of male style; D, lateral view of male genitalia.

The female last ventral segment (fig. 8, B) is concavely rounded on the posterior margin and slightly excavated either side of a rather short, broad median tooth, which extends beyond the posterior margin of the segment. The male plates are long, triangular, and tapered to attenuated apices. The style (fig. 8, C) is elongate, somewhat broadened basally, and gradually tapered to a sharply pointed apex, which curves outward. The aedeagus (fig. 8, D) is somewhat narrowed on the apical fourth and recurved at the most dorsal point; there it divides into a pair of slender processes, which are directed ventrally. The pygofer bears a conspicuous spine on the posterior margin at about its middle.

Geographical Range. According to known records, *Friscananus rupinatus* occurs only in California.

Distribution and Food Plants in California. Large populations of *Friscananus rupinatus* were taken during the summer of 1945 on bracken, *Pteridium aquilinum* var. *lanuginosum* near Montara, San Mateo County; but during the autumn, when bracken became dry, few adults were taken. The adults also were captured in small numbers during the spring, summer, and autumn each year from 1941 to 1945 on monkey-flower, *Diplacus aurantiacus*.

FRISCANANUS RUPINATUS VAR. BRUNNEUS N. VAR.

Friscananus rupinatus var. *brunneus* n. var. resembles *F. rupinatus* in form and general appearance but lacks the black spots at the apex of the vertex. It is 4.5–5.0 mm long.

The vertex is bluntly and angularly produced, about one and one third times as wide between the eyes at the middle as the median length in the female, and a little broader proportionately in the male.

The color is pale with reddish-brown markings. The male has two small brown spots at the apex of the vertex, a pale brownish area on basal half between the eyes. Its pronotum is pale, marked with dark brown, with a median pale spot on the anterior margin and a median portion on the posterior margin. Its scutellum is pale with dark-brown spots in the basal angles. Its elytra are subhyaline with brownish markings on clavus and disk. The apical veins and the anterior cross veins of the apical cells are dark brown. Its face is pale with pale fuscous arcs on each side.

The female has a large brownish spot on either side of the apex of the vertex and a rather broad brownish band between the eyes on the basal portion of the vertex. Its pronotum is brown with a median pale light spot on anterior margin. Its scutellum is pale brown except a median pale spot and dark-brown spots in the basal angles. Its elytra are reddish brown, subhyaline; the apical veins are dark brown, the claval and anterior veins are pale; the anterior two thirds have dark-brown areas between the pale veins. Its face is pale with darker arcs than in the male.

Genitalia: The female last ventral segment has a narrow, median, sunken spatulate process as in *Friscananus rupinatus*. The male genitalia are as in *F. rupinatus*.

Holotype female, allotype male, and female paratypes were collected on bracken near Montara, California, by H. H. P. Severin.

Distribution and Food Plants in California. Mixed populations of *Friscananus rupinatus* var. *brunneus* and *F. rupinatus* were collected on bracken, *Pteridium aquilinum* var. *lanuginosum*, growing on the Montara Mountains along the Pacific Coast.

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**TRANSMISSION OF CALIFORNIA ASTER-YELLOWS
VIRUS BY LEAFHOPPER SPECIES IN
THAMNOTETTIX GROUP**

HENRY H. P. SEVERIN

TRANSMISSION OF CALIFORNIA ASTER-YELLOWS VIRUS BY LEAFHOPPER SPECIES IN *THAMNOTETTIX* GROUP¹

HENRY H. P. SEVERIN²

INTRODUCTION

ACCORDING to Ball (1936),³ the tree- and shrub-inhabiting leafhoppers have been referred in the past to the genus *Thamnotettix*, but are widely separated from the type of the genus and belong to a number of distinct genera. He divided the genus into nine genera.

Some years ago three leafhopper species (Severin, 1929, 1934) and a biological race (Severin, 1940) of one of these species were reported to transmit California aster-yellows virus. In recent papers (Severin, 1945, 1946, 1947a, 1947b) thirteen more species have been added to the list of vectors of this virus. The present paper deals with nine species and one variety of leafhoppers in the *Thamnotettix* group, two of which have been previously recorded in the literature (Severin, 1934). All were tested for transmission of California aster-yellows virus and some for transmission of the viruses of curly top and Pierce's disease of the grapevine. The companion paper in this issue (DeLong and Severin, 1948) discusses the characters, distribution, and food plants of eight of these leafhopper species.

METHODS

The cages used and the methods of transferring leafhoppers in a dark chamber were the same as in previous investigations (Severin, 1930, 1931).

The food plants used in maintaining large populations of the geminate leafhopper, *Colladonus geminatus* (Van Duzee), and the mountain leafhopper, *C. montanus* (Van Duzee), have been recorded in previous papers (Severin, 1934, 1942). Infective *Idiodonus heidemanni* (Ball) was reared on diseased celery and asters and noninfective leafhoppers on healthy celery and asters. The other six species and the one variety in the *Thamnotettix* group were collected on their natural host plants. They were not reared on celery and asters, and no attempt was made to breed them on their natural host plants.

IDIODONUS HEIDEMANNI (BALL)

Transmission of Virus to Celery. To determine the efficiency of *Idiodonus heidemanni* (Ball) (= *Thamnotettix heidemanni* Ball) in transmitting California aster-yellows virus, 50 males and 50 females that had completed the nymphal stages on infected celery were transferred singly to healthy celery plants. As table 1 shows, 12 per cent of the males and 20 per cent of the females caused infections.

A comparison was made of the transmission of the virus by 25 lots each of 5, 10, and 20 males and females which had completed the nymphal stages on

¹ Received for publication May 23, 1947.

² Entomologist in the Experiment Station.

³ See "Literature Cited" for citations, referred to in the text by author and date.

diseased celery. The lots of 5 were kept on the first set of healthy celery plants to which they were transferred. With the lots of 10 and 20 leafhoppers, if symptoms developed on a plant, the lot on that plant was transferred to another healthy celery plant. If there were no symptoms, some lots were kept on the first healthy celery plant during adult life; others were changed monthly to successive healthy plants until the last adult died. These results appear in table 1.

TABLE 1
TRANSMISSION OF VIRUS BY VARYING NUMBERS OF *Idiodonus heidemanni*
TO SUCCESSIVE HEALTHY CELERY PLANTS

Number of adults in each lot	Number of lots	First set of celery			Second set of celery			Third set of celery		
		Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
1 male.....	50	50	6	12
1 female.....	50	50	10	20
5 males.....	25	25	5	20
5 females.....	25	25	8	32
10 males.....	25	25	18	72	13	4	31	2	1	50
10 females.....	25	25	16	64	16	10	63	6	3	50
20 males.....	25	25	19	76	22	12	55	10	4	40
20 females.....	25	25	16	64	20	17	85	14	5	36

Number of adults in each lot	Number of lots	Fourth set of celery			Fifth set of celery			Total		
		Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
1 male.....	50	50	6	12
1 female.....	50	50	10	20
5 males.....	25	25	5	20
5 females.....	25	25	8	32
10 males.....	25	1	0	0	41	23	56
10 females.....	25	2	0	0	49	29	59
20 males.....	25	1	0	0	1	0	0	59	35	59
20 females.....	25	8	5	63	4	1	25	71	44	62

Table 1 shows that the relation between the percentages of infection caused by males and females, and by lots of 10 and 20, was not constant in the first four sets of celery plants. But the total percentages of infections caused by single males, and by lots of 5, 10, and 20 males, were lower than those of the females. In the lots of 10 and 20, the females inoculated more plants and lived longer than the males. There was a progressive increase in the total percentages of infections with lots of 5, 10, and 20 adults. Thus the number of leafhoppers plays an important role in the transmission of the virus to celery plants.

Transmission of Virus to Asters. Fifty infective male and 75 female *Idiodonus heidemanni* were kept singly on healthy asters until symptoms developed, or during adult life if no symptoms appeared. Fourteen per cent of the males and 8 per cent of the females (table 2), an average of 13 per cent, transmitted the virus to asters, as compared with an average of 16 per cent to celery (table 1).

An experiment was conducted with lots of 5, 10, and 20 males and females to determine the percentages of transmission of the virus to successive sets of healthy asters. Each lot of infective adults was kept on a healthy aster until symptoms of the disease appeared and then was transferred to another healthy aster. If no symptoms developed, the surviving adults were changed to successive asters at irregular intervals until the last adult died. The results obtained appear in table 2.

TABLE 2
TRANSMISSION OF VIRUS BY VARYING NUMBERS OF *Idiodonus heidemanni*
TO SUCCESSIVE HEALTHY ASTERS

Number of adults in each lot	Number of lots	First set of asters			Second set of asters		
		Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
1 male.	50	50	7	14
1 female.	75	75	6	8
5 males.	20	20	1	5
5 females.	20	20	15	75	14	3	21
10 males.	10	10	4	40	6	0	0
10 females.	10	10	9	90	10	5	50
20 males.	20	20	6	30	4	1	25
20 females.	23	23	11	48	16	3	19

Number of adults in each lot	Number of lots	Third set of asters			Total		
		Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
1 male.	50	50	7	14
1 female.	75	75	6	8
5 males.	20	20	1	5
5 females.	20	4	2	50	38	20	53
10 males.	10	4	0	0	20	4	20
10 females.	10	7	1	14	27	15	56
20 males.	20	1	1	100	25	8	32
20 females.	23	4	2	50	43	16	37

As table 2 shows, the total percentages of infections of the three sets of asters did not increase progressively with lots of 5, 10, and 20 adults: higher percentages of infections were obtained with lots of 5 and 10 adults than with lots of 20 adults. The total percentages of infections were higher with lots of 5, 10, and 20 females than with the males.

Transmission of Virus to Two Host Plants. Transmission of California aster-yellows virus to celery alternating with aster, and to asters alternating with celery, by lots of 20 and 40 male *Idiodonus heidemanni*, was compared. The first and second sets of plants were each exposed to the leafhoppers for a period of 3 days and the third plant during adult life of the insects.

Table 3 shows that when the first sets of celery and asters were exposed to lots of 20 and 40 males for three days, 4 of 20 celery plants and 2 of 20 asters were infected. When the second set of celery plants was exposed to the same number of leafhoppers during adult life, 17 of 20 plants were infected. When

asters alternated with celery, it is evident from table 3, celery was more readily infected than asters.

Retention of Virus by Single Adults. Virus retention was determined with single adult *Idiodonus heidemanni* that had transmitted the virus to celery

TABLE 3
TRANSMISSION OF VIRUS TO SUCCESSIVE SETS OF TWO HOST PLANTS BY 10
LOTS EACH OF 20 OR 40 MALE *Idiodonus heidemanni*

Test. no. and plants tested	20 adults per lot			40 adults per lot		
	Period of inoculation, days	Plants inoculated	Plants infected	Period of inoculation, days	Plants inoculated	Plants infected
Test 1:						
First set of celery.....	3	10	2	3	10	2
First set of asters.....	3	10	0	3	10	2
Second set of celery.....	21-59	10	8	15-60	10	9
Test 2:						
First set of asters.....	3	10	1	3	10	2
First set of celery.....	3	10	2	3	10	6
Second set of asters.....	23-34	10	1	7-18	10	1

TABLE 4
TRANSMISSION OF VIRUS TO CELERY AND ASTERS BY VARYING
NUMBERS OF *Idiodonus kirkaldyi*

Plant and number of adults in each lot	Number of lots	Plants inoculated	Plants infected	Per cent infected
Celery:				
1 male.....	50	50	0	0
1 female.....	50	50	0	0
4 females.....	1	7	2	29
5 males.....	3	3	0	0
5 females.....	3	3	0	0
10 males.....	1	1	0	0
10 females.....	3	1	0	0
20 males.....	1	1	0	0
25 males.....	1	5	0	0
Aster:				
1 male.....	11	11	0	0
1 female.....	14	14	0	0
5 males.....	2	2	0	0
5 females.....	2	2	0	0
10 males.....	1	1	0	0
10 females.....	1	1	0	0
20 males.....	1	1	0	0

in tests of vector efficiency. After a leafhopper had produced the first infection, it was transferred daily to healthy asters during adult life. One male infected the first 2 successive asters, retaining the virus for 11 days after producing the first infection. The incubation period of the disease in the first aster is not included in the retention of the virus, since the adult was able to acquire the virus again. The longevity of the male was 67 days. Two males and 2 females produced only 1 infection in the first aster.

Attempt to Transmit Curly-Top Virus. Because the beet leafhopper, *Eutettix tenellus* (Baker), was originally described in the genus *Thamnotettix*, a large number of tests were made to determine whether *Idiodonus heidemanni*, also formerly placed in the genus *Thamnotettix*, was a vector of the curly-top virus. All attempts to transmit curly-top virus to healthy sugar-beet seedlings were failures.

IDIODONUS KIRKALDYI (BALL)

Idiodonus kirkaldyi (Ball) (= *Thamnotettix kirkaldyi* Ball) (plate 1, A, B) is an inefficient vector of California aster-yellows virus. The leafhoppers tested were collected from California sagebrush, *Artemisia californica*. One lot of 4 adults was kept on diseased celery for 66 days and then was transferred every week to successive healthy celery plants until the last adult died. Two of 7 plants inoculated were infected. Fifty males and 50 females tested singly on healthy celery produced no infection. Lots of 5, 10, 20, and 25 males or females which were kept on diseased celery from 10 to 14 days, failed to transmit the virus to healthy celery plants (table 4). The virus was not transmitted from infected to healthy asters by varying numbers of adults (table 4).

GEMINATE LEAFHOPPER, COLLADONUS GEMINATUS (VAN DUZEE)

The geminate leafhopper, *Colladonus geminatus* (Van Duzee) (= *Thamnotettix geminatus* Van Duzee), was previously reported (Severin, 1934) as a vector of the California aster-yellows virus. In a later investigation (Severin, 1942), this leafhopper species was demonstrated to be one of the most important vectors of the virus to perennial delphiniums, and was shown to breed on this host plant under natural conditions.

The number of infections induced by varying numbers of adults has been reported in two previous papers (Severin, 1934, 1942). The transmission of the virus to celery averaged 14 per cent, but no infections were obtained with 182 asters inoculated. Twenty-five lots of 50 males each were used to inoculate asters; and 4 of 50 asters were infected, or 8 per cent (Severin, 1942).

Transmission of Virus to Celery and Asters. To determine the efficiency of the geminate leafhopper in transmitting the California aster-yellows virus to celery, 100 males and 100 females that had completed the nymphal stages on diseased celery were transferred singly to healthy celery plants. Two males and 3 females, or 3 per cent, produced infections.

Twelve lots of 20 males were kept on diseased celery for 5, 10, or 15 days, and then each lot was transferred to 37, 32, or 27 successive healthy celery plants. The average number of infections caused by lots of 20 adults was 2.4, 1.5, and 2.4, with exposures of 5, 10, and 15 days, respectively.

Another experiment was undertaken with asters as the host plant. Three hundred males and 300 females that had completed the nymphal stages on naturally infected asters and on experimentally infected asters and celery, were transferred singly to healthy asters. Not a single infection was obtained.

As reported in a previous paper (Severin, 1934), the geminate leafhopper was collected on asters under natural conditions; but a high mortality occurred on small asters in the greenhouse when the adults were transferred

from large asters in the field. It was found that the adults fed on small healthy asters died within a week. Nymphs lived longer than adults, and sometimes the nymphs acquired the winged stage on large asters.

Latent Period of Virus in Adults. The latent period of California aster-yellows virus in the geminate leafhopper was determined with 8 lots of single previously noninfective males and 11 lots of 100 males. After 1 day on an

TABLE 5
LATENT PERIOD OF VIRUS IN ADULT GEMINATE LEAFHOPPER, *Colladonus*
geminatus, WITH CELERY AS THE HOST PLANT

Lot no.	Number of adults	Days on infected celery	Successive plants inoculated	Plants infected	Per cent infected	Days on which successive infections occurred, including initial day on infected celery	Adults alive at end of 42 days
1*	1	1	41	1	3	31	1
9	100	1	41	8	20	18, 26, 31, 34, 36, 39, 41, 42	62
10	100	1	41	12	29	21, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41	75
11	100	1	41	8	20	23, 26, 27, 28, 30, 32, 39, 41	62
12	100	1	41	7	17	24, 25, 26, 27, 28, 29, 30	62
13	100	1	41	2	5	27, 37	37
14	100	1	41	13	32	27, 28, 30, 31, 32, 34, 35, 36, 37, 38, 39, 41, 42	48
15	100	1	41	9	22	31, 32, 34, 35, 36, 37, 38, 39, 42	69
16	100	1	41	5	1	32, 34, 35, 39, 42	15
17	100	1	41	3	7	33, 37, 42	57
18	100	1	41	5	1	36, 37, 38, 39, 41, 42	69
19	100	1	41	6	2	36, 37, 39, 41, 42	48
20	20	5	37	2	6	22, 23	8
21	20	5	37	1	3	22	10
22	20	5	37	1	3	23	9
23	20	5	37	3	8	26, 30, 34	10
24	20	5	37	2	6	27, 31	7
25	20	10	32	1	3	17	7
26	20	10	32	1	3	33	9
27	20	15	27	2	7	20, 29	10
28	20	15	27	2	7	23, 33	11
29	20	15	27	1	4	31	5
30	20	15	27	1	4	33	8
31	20	15	27	1	4	38	20

* Lots 2 to 8 were single-insect tests; no infections were obtained with them.

infected celery plant, the adults were transferred daily to successive healthy celery plants throughout a period of 41 days. The virus latent period in 1 male was 31 days, as appears in table 5. Seven lots of single adults kept on diseased celery plants for 1 day and then transferred daily to healthy celery during 41 days failed to cause infections, but 2 adults kept on healthy celery produced infections after 54 and 90 days (not included in table 5). The minimum latent period in 11 lots of 100 males ranged from 18 to 36 days.

Attempts to Transmit the Viruses of Curly Top and Pierce's Disease of the Grapevine. In discussing the number of leafhopper vectors of the California aster-yellows virus, plant pathologists have asked repeatedly whether any of these leafhoppers transmit the viruses of curly top and Pierce's disease of the grapevine. All efforts to transmit the curly-top virus by means of the geminate leafhopper were failures. The geminate leafhopper also failed to

transmit the virus of Pierce's disease to healthy wild grapevine seedlings and to California common or Chilean alfalfa; and from alfalfa dwarf to healthy wild grapevine seedlings and healthy alfalfa.

MOUNTAIN LEAFHOPPER, *COLLADONUS* *MONTANUS* (VAN DUZEE)

The mountain leafhopper, *Colladonus montanus* (Van Duzee) (= *Thamnotettix montanus* Van Duzee), has been previously reported as a vector of the California aster-yellows virus (Severin, 1934). This leafhopper is an efficient vector of the virus to perennial delphinium and breeds on this host plant under natural conditions (Severin, 1942).

The number of infections produced by lots of 5, 10, and 20 adults has been published in a previous paper (Severin, 1934). A total of 464 celery plants and 412 asters were inoculated; 121 celery plants and 12 asters were infected, or 26 and 3 per cent, respectively.

Transmission of Virus to Celery and Asters. Fifty males and 50 females that had completed the nymphal stages on diseased celery were tested singly on healthy celery plants to determine the efficiency of the mountain leafhopper in transmitting the California aster-yellows virus. Three males and 8 females, or 11 per cent, caused infections.

Thirteen lots of 20 males kept on infected celery for 1 day and then transferred to healthy celery for 41 days caused no infections. With exposures of 5, 10, and 15 days to infected celery, the average number of infections produced by 13 lots of 20 adults was 4, 1.3, and 3, respectively. One lot of 20 adults kept on diseased celery for 5 days and 2 lots for 10 days failed to transmit the virus to healthy celery plants.

The first experiment was repeated with asters as a host plant. Fifty males and 50 females reared during all nymphal stages on naturally infected asters, and on experimentally infected asters and celery, were transferred singly to healthy asters. No infections were produced. The longevity of the adults on asters ranged from 2 to 15 days, with an average of 4.3 days.

Latent Period of Virus in Adults. The latent period of the California aster-yellows virus in the mountain leafhopper was determined with 8 lots of single previously noninfective males and 10 lots of 100 males. All lots were kept on diseased celery plants for 1 day and then were transferred daily to successive healthy celery plants for 41 days. As shown in table 6, the latent periods of the virus in 2 single males were 23 and 31 days. Six lots of single males failed to transmit the virus. The minimum latent period of the virus in 10 lots of 100 males ranged from 8 to 40 days. The number of infections produced by each lot and the number of adults alive at the end of 42 days appear in table 6.

Retention of Virus. The retention of the California aster-yellows virus in the mountain leafhopper was determined with single males and females that had transmitted the virus in testing the efficiency of virus transmission by this vector. Each leafhopper was kept on a healthy celery plant until the latter showed symptoms, then the insect was transferred to successive healthy celery plants throughout its adult life. Two males and 1 female produced only the initial infection.

TABLE 6
LATENT PERIOD OF VIRUS IN ADULT MOUNTAIN LEAFHOPPER, *Colladonus montanus*, WITH CELERY AS THE HOST PLANT

Lot no.	Number of adults	Days on infected celery	Successive plants inoculated	Plants infected	Per cent infected	*Days on which successive infections occurred	Adults alive at end of 42 days
1	1	1	41	1	3	23	1
2	1	1	41	1	3	31	1
3	100	1	41	7	17	8, 11, 18, 28, 37, 39, 41	42
4	100	1	47	11	27	15, 16, 17, 20, 22, 26, 27, 29, 32, 35, 41	57
5	100	1	47	8	20	20, 35, 36, 37, 38, 40, 41, 42	45
6	100	1	41	3	7	20, 29, 32	40
7	100	1	41	9	22	23, 25, 26, 28, 30, 33, 35, 36, 41	78
8	100	1	41	13	32	24, 26, 27, 28, 29, 30, 31, 32, 34, 35, 37, 38, 42	61
9	100	1	41	11	27	26, 29, 30, 31, 32, 33, 34, 35, 38, 40, 42	60
10	100	1	41	3	7	30, 32, 35	15
11	100	1	41	1	3	30	4
12	100	1	41	2	5	40, 42	73
13	20	5	37	3	8	22, 32	17
14	20	5	37	9	3	23, 27, 29, 34, 35, 36, 38, 40, 42	6
15	20	5	37	2	6	23, 24	14
16	20	5	37	2	6	33, 36	8
17	20	10	32	1	3	19	15
18	20	10	32	1	3	27	19
19	20	10	32	1	3	32, 35	14
20	20	15	27	3	1	19, 30, 36	17
21	20	15	27	3	1	28, 38, 42	16
22	20	15	27	4	2	29, 30, 34, 37	20
23	20	15	27	4	2	29, 32, 35, 36	17
24	20	15	27	3	1	29, 39, 41	14
25	20	15	27	1	3	38	20

* Days are numbered from the initial day on infected celery.

TABLE 7
TRANSMISSION OF VIRUS TO CELERY AND ASTERS BY VARYING
NUMBERS OF *Colladonus commissus*

Plant and number of adults in each lot	Number of lots	Plants inoculated	Plants infected	Per cent infected
Celery:				
1 male.....	50	50	14	28
1 female.....	50	50	24	48
10 males.....	1	13	9	69
20 males.....	1	9	2	22
25 males.....	3	24	6	25
25 females.....	2	56	16	28
Asters:				
1 male.....	1	1	0	0
1 female.....	8	8	0	0
5 females.....	5	5	1	20

Attempts to Transmit the Viruses of Curly Top and Pierce's Disease of the Grapevine. The mountain leafhopper failed to transmit curly-top virus. It also failed to transmit the virus of Pierce's disease to healthy wild grapevine seedlings and to California common or Chilean alfalfa; and from alfalfa dwarf to healthy wild grapevine seedlings or to healthy alfalfa.

COLLADONUS COMMISSUS (VAN DUZEE)

Colladonus commissus (Van Duzee) (= *Thamnotettix commissus* Van Duzee) (plate 1, C, D,) was collected on monkey-flower, *Diplacus aurantiacus*.

Transmission of Virus to Celery. The efficiency of *Colladonus commissus* in transmitting the California aster-yellows virus was determined with single adults, each transferred from a diseased to a healthy celery plant. Table 7 shows that 14 to 50 males and 24 of 50 females caused infections of celery plants.

TABLE 8
RETENTION OF VIRUS BY SINGLE ADULT *Colladonus commissus*
WITH CELERY AS THE HOST PLANT

Days on first plant before symptoms developed	Plants inoculated after first infection	Plants infected after first infection	Per cent infected after first infection	Days after first infection on which successive infections occurred	Longevity of adults, days
23	24	12	50	3, 4, 5, 8, 9, 10, 12, 13, 14, 15, 18, 27	47
21	39	3	8	2, 4, 6	60

Lots of 10 to 25 males or females were transferred from diseased to successive healthy celery plants weekly. Table 7 shows the number of infections produced by each lot of leafhoppers.

To determine whether a longer period of exposure to healthy celery plants is a factor in the transmission of the virus, a male and a female were transferred singly from diseased to successive healthy celery plants every month during adult life. The male infected 2 of 3 plants and the female 3 of 4 plants.

Transmission of Virus to Asters. No infections were obtained with 9 adults tested singly on asters. Five lots of 5 adults transmitted California aster-yellows virus to 1 of 5 asters (table 7).

Retention of Virus by Single Adults. The retention of California aster-yellows virus by *Colladonus commissus* was determined with single adults that had transmitted the virus in tests of vector efficiency. After the first infection of celery, the leafhopper was transferred daily to successive healthy celery plants during adult life. One female retained the virus for 27 days after symptoms developed on the first plant and caused 12 infections, as appears in table 8. Another female retained the virus for 6 days and produced 3 infections. The period on the first plant is not included in the retention of the virus since the adults were able to acquire the virus again. Eleven adults induced only the initial infection.

Attempts to Transmit the Viruses of Curly Top and Pierce's Disease of the Grapevine. Because of the interest in curly top and Pierce's disease of the grapevine, attempts were made to transmit these by means of *Colladonus commissus*.

There was no proof that *Colladonus commissus* could transmit the curly-top virus to healthy sugar-beet seedlings or the virus of Pierce's disease of the grapevine to healthy wild grapevine seedlings. Some of the beet seedlings showed cleared veinlets on a portion of a leaf, a reliable symptom of curly top; but noninfective beet leafhoppers failed to recover the curly-top virus from such plants.

COLLADONUS MENDICUS (BALL)

Colladonus mendicus (Ball) (= *Thamnotettix mendicus* Ball) has not been demonstrated to be a vector of California aster-yellows virus. One lot of 32 adults collected on creek nettle, *Urtica gracilis* var. *holosericea* on October 27, 1942, was kept on diseased celery for 19 days, and then 2 lots of 5 and 15 adults that survived were changed to 2 healthy celery plants, without results. Another lot of 5 adults, captured on California blackberry, *Rubus vitifolius*, was fed on an infected celery plant for 34 days and then transferred to a healthy celery plant, but caused no infection. One lot of 10 adults collected on creek nettle was kept on a diseased aster for 11 days; 4 surviving adults, transferred to a healthy celery plant, caused no infection. The longevity of the adults on healthy celery was only 4 days.

Colladonus mendicus was parasitized by a new species of Pipunculidae, described as *Allomethus oleous* Rapp (1943).

COLLADONUS FLAVICAPITATUS (VAN DUZEE)

Colladonus flavicapitatus (Van Duzee) (= *Thamnotettix flavicapitatus* Van Duzee) (plate 1, *E, F*), collected on wild gooseberry, *Ribes* sp., in General Grant Park (DeLong and Severin, 1947) was usually parasitized by a species of Pipunculidae. The parasite was not reared. The leafhoppers were killed by the parasite before the virus incubation period in the insects was completed; this happened with 39 adults collected on September 15, 1944.

The transmission of the virus by this species of leafhopper was limited to three tests. One adult, after 11 days on a diseased celery plant, was changed to 3 successive celery plants at irregular intervals of 11 to 36 days and caused 2 infections. A single male was kept on an infected celery plant for 19 days and then on a healthy celery plant during adult life, but failed to produce an infection. One lot of 10 adults was kept on an infected celery plant for 17 days and then transferred to 5 successive healthy celery plants; 2 infections resulted.

FRISCANANUS INTRICATUS (BALL)

Friscananus intricatus (Ball) (plate 1, *G, H, I*) is rare on the host plants recorded in the companion paper by DeLong and Severin (1948); but although low populations of leafhoppers were used, transmission of the virus to healthy celery was obtained.

Twenty males and females tested singly caused 3 infections (table 9). Two lots of 3 and 4 adults collected during the summers of 1943 and 1944 were kept on diseased celery from 11 to 12 days, and then were transferred to 4 and 13 successive healthy celery plants, respectively; 6 infections resulted—

35 per cent. The longevities of the last surviving adult in the two lots on healthy celery were 67 and 93 days, respectively, or an average of 80 days.

No transmissions from infected to healthy asters were obtained with 3 males and 1 female tested singly (table 9).

TABLE 9
TRANSMISSION OF VIRUS TO CELERY AND ASTERS BY VARYING
NUMBERS OF *Friscananus intricatus*

Plant and number of adults in each lot	Number of lots	Plants inoculated	Plants infected	Per cent infected
Celery:				
1 male.....	6	6	1	17
1 female.....	14	14	2	14
3 males.....	1	4	0	0
4 females.....	1	13	6	46
Aster:				
1 male.....	3	3	0	0
1 female.....	1	1	0	0

TABLE 10
TRANSMISSION OF VIRUS TO CELERY AND ASTERS BY VARYING
NUMBERS OF *Friscananus rupinatus*

Plant and number of adults in each lot	Number of lots	Plants inoculated	Plants infected	Per cent infected
Celery:				
1 male.....	50	50	15	30
1 female.....	50	50	14	28
5 males.....	3	3	1	33
5 females.....	2	2	1	50
10 males.....	1	1	0	0
15 males.....	1	9	2	22
15 females.....	1	12	0	0
15 males.....	3	3	2	67
15 females.....	6	6	2	33
Asters:				
1 male.....	8	8	0	0
1 female.....	25	25	0	0

FRISCANANUS RUPINATUS (BALL)

Nymphs and adults of *Friscananus rupinatus* (Ball) (= *Thamnotettix rupinatus* Ball) (plate 1, J, K) were collected on bracken, *Pteridium aquilinum* var. *lanuginosum*. Fifty males and 50 females, tested singly on healthy celery plants, produced 15 and 14 infections, respectively (table 10). The transmission of the virus to healthy celery by lots of 5, 10, and 15 adults is shown in table 10. Nine lots of 15 males or females kept on healthy celery plants during adult life transmitted the virus to 4 of 9 plants. During the summer of 1943, 1 lot of 15 males, after feeding on infected celery for 19 days, was transferred to 9 successive celery plants and caused 2 infections. During the summer of 1944, 1 lot of 15 females was kept on diseased celery for 12

days and then was changed to 12 successive healthy celery plants, but no infection resulted. The longevities of the last surviving adult in the two lots on healthy celery were 41 and 110 days, or an average of 76 days.

Eight males and 25 females, after feeding on infected asters 10 days or longer, were transferred to healthy asters and kept on them during adult life; but no infection occurred (table 10).

An attempt was made to transmit the curly-top virus by means of *Friscananus rupinatus*. Ten lots of 5 or 10 males or females were exposed to curly-top beets for a few days and then each lot was transferred to a healthy beet. All beets remained healthy.

FRISCANANUS RUPINATUS VAR. BRUNNEUS DE LONG AND SEVERIN

Friscananus rupinatus var. *brunneus* DeLong and Severin (plate 1, *L*) was collected on bracken. Its efficiency in transmitting California aster-yellows virus was determined with single adults, each transferred from a diseased to a healthy celery plant. Infections were produced by 3 of 10 males, or 30 per cent; and by 11 of 50 females, or 22 per cent. As compared with these percentages, 50 males and 50 females of *Friscananus rupinatus* kept singly on healthy celery infected 30 and 28 per cent, respectively (table 10).

SUMMARY

Evidence is presented in this paper that the following leafhoppers in the *Thamnotettix* group are vectors of the California aster-yellows virus:

Idiodonus heidemanni (Ball)

Idiodonus kirkaldyi (Ball)

Geminate leafhopper, *Colladonus geminatus* (Van Duzee)

Mountain leafhopper, *Colladonus montanus* (Van Duzee)

Colladonus commissus (Van Duzee)

Colladonus flavocapitatus (Van Duzee)

Friscananus intricatus (Ball)

Friscananus rupinatus (Ball)

Friscananus rupinatus var. *brunneus* DeLong and Severin

TABLE 11

SUMMARY OF RESULTS ON EFFICIENCY OF LEAFHOPPER SPECIES IN TRANSMITTING VIRUS, EACH VECTOR TESTED SINGLY ON HEALTHY CELERY OR ASTERS

Species of leafhopper	Number of lots	Celery			Asters		
		Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
<i>Idiodonus heidemanni</i>	100	100	16	16	125	13	13
<i>Idiodonus kirkaldyi</i> *.....	100	100	0	0	25	0	0
<i>Colladonus geminatus</i>	200	200	5	3	300	0	0
<i>Colladonus montanus</i>	100	100	11	11	100	0	0
<i>Colladonus commissus</i>	100	100	38	38	9	0	0
<i>Colladonus flavocapitatus</i>	2	4	3	75
<i>Friscananus intricatus</i>	20	20	3	15	4	0	0
<i>Friscananus rupinatus</i>	100	100	29	29	33	0	0
<i>Friscananus rupinatus</i> var. <i>brunneus</i>	60	60	14	23

* Infection was obtained with multiple lots.

A summary of results on the efficiency of each leafhopper species in transmitting the virus when tested singly on healthy celery or asters is given in table 11.

Celery is more readily infected than asters. The number of leafhoppers and the period of exposure to healthy plants plays an important role in the transmission of the virus to celery plants.

The latent period of the virus in 1 male of the geminate leafhopper, *Colladonus geminatus*, was 31 days. The minimum latent period in 11 lots of 100 males ranged from 11 to 36 days. In the mountain leafhopper, *C. montanus*, the minimum latent period in 10 lots of 100 adults ranged from 8 to 40 days.

The retention of the virus by 1 male *Idiodonus heidemanni* was 11 days after producing the initial infection; 2 males and 2 females each caused infection only in the first celery or aster. In tests on the retention of the virus with the mountain leafhopper, *Colladonus montanus*, 2 males and 1 female produced only the initial infection. One female of *C. commissus* retained the virus for 6 days and produced 3 infections. Eleven adults induced only the initial infection.

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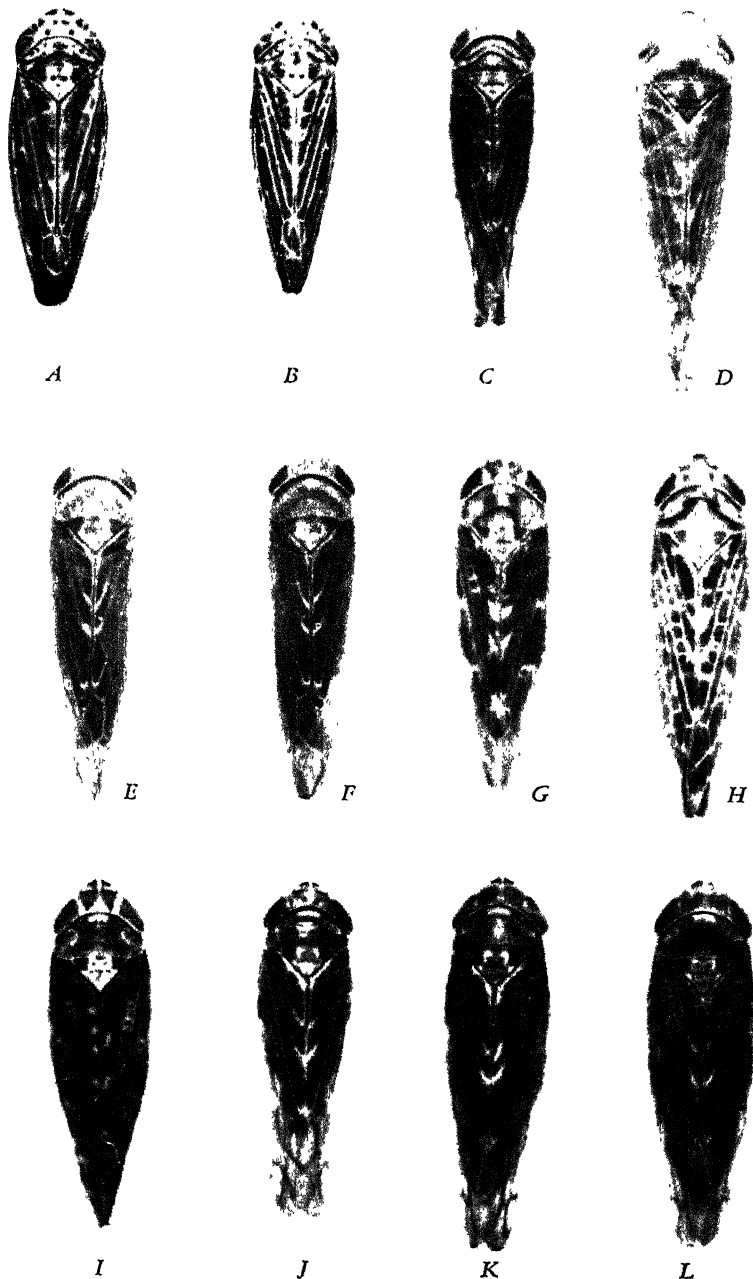


Plate 1.—Color patterns which usually distinguish species of leafhoppers in *Thamnottetia* group, vectors of California aster-yellows virus: A, male, and B, female, *Idiodonus kirkaldyi* (Ball); C, male, and D, female, *Colladonus commissus* (Van Duzee); E, male, and F, female, *C. flavocapitatus* (Van Duzee); G, male, and H, I, females, *Friscananus intricatus* (Ball); J, male, and K, female, *F. ruginatus* (Ball); L, *F. ruginatus* var. *brunneus* DeLong and Severin.

PHLOEM STRUCTURE IN THE GRAPEVINE, AND ITS SEASONAL CHANGES¹

KATHERINE ESAU²

INTRODUCTION

THE PRESENT INVESTIGATION of the phloem of the grapevine, *Vitis vinifera* L., was undertaken to provide a background for a study of the anatomical effects of the virus-induced Pierce's disease upon this plant. According to the previous studies of Pierce's disease (Butler, 1910; Houston *et al.*, 1947),³ the xylem appears to be the tissue affected primarily, but the irregularity in the maturation of the bark suggests a possible disturbance in the phloem also. The close relation of the causal agent of Pierce's disease to the xylem places this virus in an uncommon category. With regard to their tissue relationships, viruses have been divided, thus far, into those that are largely restricted to the phloem and those that are not so restricted and invade various types of parenchyma (Bennett, 1940). To determine whether Pierce's disease affects the phloem at all, a detailed study of this tissue in healthy and diseased vines was carried out. The present paper deals with the phloem of plants that showed no symptoms of any disease.

A study of the grapevine phloem promised to be of interest also from the standpoint of phloem anatomy in general. The phloem of *Vitis* shows an unusually long life. Its sieve tubes function more than one year, becoming inactive during the winter and resuming activity in the spring. Though the general structure and development of the grape phloem was thoroughly investigated by Wilhelm (1880), the phenomenon of reactivation of this tissue seemed to merit a renewed consideration in the light of the modern concepts of phloem structure. Moreover, it seemed that information relating the seasonal changes in the phloem to the various manifestations of growth and reproduction in the plant would further explain the connection between structure and function of the sieve tubes.

REVIEW OF LITERATURE

The histology of the grapevine phloem and its various transformations have been considered by Wilhelm (1880), Strasburger (1891), and Hill (1908) and the findings of these workers are evaluated throughout the present study. However, information on seasonal changes in secondary phloem and the rela-

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³ See "Literature Cited" for citations, referred to in the text by author and date.

tion of these changes to cambial activity is rather fragmentary in the individual papers, and the data require a separate review at this time.

Judging from the literature (see review by Esau, 1939), the secondary phloem of most plants shows only one period of activity. After the sieve tubes reach maturity,⁴ they remain in this state for periods of time which vary mainly in relation to the plant species; the sieve tubes then become senile and functionless. As senility approaches, the callus⁵ on the sieve areas thickens until it reaches its maximum development. This accumulation of callose constitutes the definitive callus whose presence signifies that the sieve tube is no longer concerned with the primary activity of the phloem, the conduction of food. The definitive callus usually disappears later, but the sieve tubes then have no living contents and may become filled with gases, if they are not crushed.

Commonly the sieve tubes function only one season. (See reviews by Strasburger, 1891; Esau, 1939.) Recently Schneider (1945) considered the phloem of *Prunus* (peach and cherry) from a developmental standpoint and also found that a given phloem increment lost its activity at the end of the first season. Few exceptions to such behavior have been recorded for angiosperms. Janczewski (1881) considered that the same sieve tubes functioned for four seasons in *Tilia*. He based his estimate on the assumption that each season two tangential bands containing sieve tubes (and alternating with fiber bands) were produced. Hence, finding eight bands with active sieve tubes, he concluded that the outermost were in the fourth season of their function. Though such an estimate is obviously unreliable—the number of tangential bands produced during one season could be expected to vary—Janczewski reported also that the winter sieve tubes did not appear functionless in *Tilia*. Lecomte (1889) stated that the sieve tubes of the rose functioned two years. According to Lodewick (1928), the sieve tubes of *Liriodendron* remained active possibly for two years, though in several other forest trees, which he considered, these elements showed the usual loss of function at the end of the first season.

As mentioned in the introduction, *Vitis* contrasts with most plants in having sieve tubes that pass through more than one period of activity (Wilhelm, 1880; Strasburger, 1891; Lecomte, 1889; Hill, 1908). Before the onset of winter dormancy, the *Vitis* sieve tubes develop provisional (dormancy) callus. Their protoplasts⁶ do not die, however, and in the spring the sieve tubes again acquire the same characteristics of active elements that they showed when they first differentiated from the cambium. In the present paper, the term *reactivation* designates this phenomenon of resumption of activity by sieve tubes that have passed through a period of dormancy. Wilhelm (1880) implied that reactivation could occur more than once in the same sieve tubes.

One of the outstanding features of the reactivation process is the removal of the dormancy callus. This phenomenon is not identical with the disappearance of the definitive callus in functionless sieve tubes. When the definitive callus is gone, no callose at all remains on the sieve plate: the latter is then

⁴ For definition of maturity in sieve tubes, see Esau, 1947.

⁵ *Callus* is used here, as in most literature on phloem (see review by Esau, 1939), to designate the more or less large deposits of callose on the sieve areas.

⁶ Although a mature sieve-tube element has no discrete nucleus, the substance contained within such an element is here designated as "protoplast" because it continues to exhibit some properties of an organized protoplasmic unit after the disintegration of the nucleus.

simply a cellulose net with openings that were formerly occupied by the callus cylinders encasing the connecting strands (Esau, 1938, 1939). When the provisional callus disappears, the sieve plate shows no free openings: the callus cylinders and the included connecting strands remain within the sieve-plate pores (Wilhelm, 1880; Hill, 1908).

Few other genera besides *Vitis* have been named in the literature as having more than one period of activity and, with regard to the phenomenon of reactivation, none were investigated in such detail as the grapevine. Janczewski (1881) mentioned that the sieve tubes of *Phragmites*, and apparently those of other monocotyledons, were "reopened in the spring." He also found, in *Tecoma*, that the winter contents of the sieve tubes (whose sieve plates were calloused over) were in such a state that they could be considered capable of resuming activity. Lecomte (1889) described the reactivation of sieve tubes as a general characteristic of the perennial monocotyledons. He stated that in such plants the sieve tubes, presumably not those of the protophloem, remained alive nearly as long as the organ in which they occurred. During the winter these sieve tubes showed increased amounts of callose, but in the spring the latter was reduced to its original thickness.

The occurrence or nonoccurrence of phloem reactivation bears upon the question of the relative time when activity is resumed in the spring by the cambium and by the phloem. If phloem reactivation takes place, does it begin before or after the cambium becomes active? If the phloem is not reactivated, is any functioning phloem present when cambial divisions begin? If such phloem is available, what is its origin? Does some mature phloem remain in an active state through the winter or does some immature phloem differentiate in advance of cambial reactivation? Or does the cambium become active in the absence of any functioning phloem?

The first question is not answered in the literature. The students of *Vitis* phloem did not consider the cambium (Wilhelm, 1880; Strasburger, 1891; Hill, 1908), whereas Knudson (1916), who studied radial growth in *Vitis labrusca* L., did not investigate the phloem.

With regard to plants having no reactivation, the literature previously reviewed by the writer (Esau, 1939, p. 419-21) and certain other references (Knudson, 1913; Huber, 1939) suggest that, if in the given species the phloem functions only one season, some cambial derivatives on the phloem side pass the winter in an undifferentiated state and complete their maturation in the spring before the cambium resumes its activity. Occasional narrow sieve tubes formed at the end of a season may be carried over into the second season (Huber, 1939). Reports on gymnosperms are similar to those on angiosperms except that, according to Huber (1939), in the Abietineae part of a given phloem increment is not crushed until the second season.

In plants with sieve tubes that function for several years and develop no provisional callus during the winter, some sieve tubes can be expected to be ready for function in the spring as soon as the growing season begins.

In contrast to the scarcity of information on the beginning of phloem activity in the spring, there is an abundant literature on cambial reactivation. Furthermore, this topic was previously reviewed in the literature as, for example, by Grossenbacher (1915) and Priestley (1930). Since these reviews

were written, a growth hormone was found to be one of the agents inducing cambial divisions in the spring, and this discovery has much clarified the picture of cambial reactivation (Avery *et al.*, 1937). Thus, the commonly observed inception of cambial growth beneath the buds and the basipetal progression of this growth through the branches and trunk toward the root is now, at least partially, explained by the observation that the growth substance is formed in buds emerging from winter dormancy and then appears in measurable amounts at successively lower levels of the tree in advance of cambial reactivation (Avery *et al.*, 1937).

Because of the basipetal progress of cambial activity, the root usually begins radial growth considerably later than the aerial parts of the plant. As an example, Cockerham's (1930) observations on *Acer pseudo-platanus* L. may be cited. In this tree, nine to ten weeks elapse between the inception of xylem differentiation in the twigs (late in April) and that in the proximal portions of roots (early in July). Cessation of activity occurs in the same order and, again citing Cockerham, in *Acer* the formation of xylem ceases in the twigs late in July and in the roots late in September. Thus the period of inactivation lasts eight to nine weeks. In conifers the relation between the buds and the reactivation of cambium seems to be less obligate than in the hardwoods (Priestley, 1930; Wight, 1933).

The morphologic details of cambial reactivation have been treated in some references (Brown, 1915; Cockerham, 1930; Knudson, 1913; Lodewick, 1928; Priestley, 1930; Priestley *et al.*, 1933). In the first phase of resumed cambial activity, a radial enlargement of the meristematic cells occurs. In the second phase, cell division takes place. Priestley and his co-workers (1933) describe cambial reactivation in some detail. Assuming that the dormant cambium is a single layer of cells, they characterize these cells as being as "tough and resistant as any of the layers between the lignified wood and the bark." The first sign of resumption of cambial activity is a "swelling" of the cells in the cambial layer. In other words, the cells enlarge, and since the enlargement is in the radial direction, the radial walls become thinner, while the contents of the cells seem to change from a "solid to a liquid consistency." As soon as this stage is reached, the cambial walls break readily when the stem is handled, and the bark "slips" over the wood. After the divisions are initiated in the cambium, the bark continues to slip along the cambial layer. According to Lodewick (1928), the dormant cambium cells have rather dense protoplasts and the ray initials contain starch; when cambial activity is resumed, this starch disappears.

Reports vary regarding the relative time when the first xylem and phloem are formed by the cambium (Brown, 1915; Cockerham, 1930; Knudson, 1913, 1916; Lodewick, 1928; Priestley, 1930; Priestley *et al.*, 1933). A proper evaluation of these reports is difficult because writers usually make no clear distinction between the maturation of cells left over from the cambial activity of the previous season and the differentiation of cells formed by the new growth. The various patterns of radial growth suggested in the literature are: (1) that the formation of xylem begins before that of the phloem; (2) that the formation of xylem and that of phloem begin and cease at the same time (*Vitis labrusca* L. follows this pattern, according to Knudson, 1916); (3) that phloem

initiation follows that of the xylem and lasts longer; (4) that more cells are cut off on the xylem than on the phloem side.

Judged from the literature just reviewed, the information on the seasonal cycle of secondary phloem in woody species may be tentatively summarized as follows. Plants fall into two major groups regarding longevity of the phloem: (1) plants whose phloem functions one season only; (2) plants whose phloem functions more than one season. In the second group two further subdivisions can be made. In one, the phloem does not become inactive during the winter; in the other, it becomes dormant and is reactivated in the spring. In the much larger group of plants whose phloem functions only one season, all the mature phloem becomes functionless at the end of this season. There are, however, some immature cambial derivatives on the phloem side of the cambium. These differentiate into functioning phloem at the start of the growth season. In the plants with long-lived phloem, active sieve tubes are present when spring growth begins. These sieve tubes are active either because their structure remains unchanged during the winter or because they become reactivated in the spring. In all plants new phloem is added by the cambium in the presence of functioning phloem of earlier origin.

MATERIAL AND METHODS

The material used for the present study was obtained from grapevines that were growing under field conditions at Davis, California. The most complete collection was made from the Sultanina (Thompson Seedless) variety; for certain studies other varieties were sampled. The vines used for the samplings were several years old. Material of different ages, including some from the main trunks, was examined, but the most complete study was carried out on canes in their second year of growth. In 1945 the collections were made every week, sometimes more than once during the same week; in 1946, at fortnightly intervals. Root and stem phloem were compared. Lateral roots $\frac{1}{4}$ to $\frac{3}{4}$ inch thick were sampled 15 times during 1945 from the Sultanina variety.

In 1945 the material from each collection was used partly in fresh state and partly in preserved condition. In 1946 all of the material was killed before it was sectioned. Two formalin-acetic-alcohol mixtures, one prepared according to the formula No. 1 of Rawlins (1933), the other according to Sass (1940, p. 16), and the chrome-acetic-formalin combination Craf III (Sass, 1940, p. 19) were the killing solutions employed. The latter was the most satisfactory of the three for the study of cytological details in the sieve tubes. After a fixation of several days, the material was rinsed in alcohols and then preserved in 70 per cent alcohol.

The fresh material was sectioned on a sliding microtome and examined either fresh or after treatment with I_2KI and aniline blue (Foster, 1942, p. 142). Plates 4, A; 12; 13; 14, A to C, and E; 15; and 19, D, exemplify material treated in this manner. The killed material was cut on a sliding microtome either directly, if the pieces were sufficiently large, or after it was encased in diglycol stearate (Diglycol Stearate S of the Glyco Products Co. Inc.), a wax that is dispersible in hot water and soluble in hot alcohol. Some of the material was imbedded in paraffin after a dehydration with tertiary butyl alcohol; it was then sectioned on the sliding microtome.

To prevent curling of sections obtained by the use of the sliding microtome, the block to be cut was covered with a small piece of thin wet paper as recommended by Varrelman (1931). As the knife passed across the block, the paper slid onto the knife together with the section. The paper and the adhering section were picked off the knife with a pair of forceps and the section was floated off in water or alcohol. If the sections were obtained from paraffin material, they were placed directly on a slide covered with Haupt's adhesive and flooded with a 4 per cent formalin solution (Johansen, 1940, p. 20). Most of the sections were cut 20 microns thick; some were thinner, others thicker.

The sections from the killed material were stained either in iodine and aniline blue or in a combination of three stains: Bismark brown, iodine green, and resorcin blue. This combination proved very useful for a detailed study of the sieve plates because it clearly differentiated between the callose and the connecting strands. In killed material preserved in alcohol the callose stains very well. With the aniline-blue technique, the slime, the cytoplasm, and the callose are distinguished by different tones of blue. With the three-stain combination, the callose is blue and the connecting strands brownish purple. The callose is also well differentiated from the cellulose wall, which acquires different shades of yellow and brown (the Bismark-brown staining). The iodine green is described in the literature as a stain for chromatin and for lignified walls (Johansen, 1940, p. 59). The latter are supposed to become green in this dye. The iodine green used in the present study—an old noncertified sample—gave the lignified walls a purplish color. This stain was employed not for its effect upon walls, however, but to stain the slime and to contrast the latter with the callose. Plate 20 shows how sharply the sieve-tube slime may be differentiated, in all its states, by the use of iodine green. (No resorcin blue was used in plate 20, A.)

Plate 1 illustrates the color contrasts obtained by staining with Bismark brown, iodine green, and resorcin blue. This combination is effective not only in color photography but also in the preparation of black and white photomicrographs. (See, for example, plates 7, 10, 11, and 16.)

The schedule for the use of the Bismark brown-iodine green-resorcin blue method of staining is given in detail in the following paragraphs. Most of the material stained by this method was sectioned by the use of the diglycol stearate. Since the textbooks on microtechnique (Johansen, 1940, p. 22-23; Sass, 1940, p. 99) give few suggestions for the use of this "encasing" medium, the method employed in the present study is described in connection with the staining procedure. The combination of the two schedules is as follows.

The material, previously stored in 70 per cent alcohol, is passed through two or three grades of lower alcohols to distilled water. It is left in distilled water overnight, since the material is too brittle if sectioned directly out of the storage alcohol.

The stem pieces are then removed from the distilled water, wiped off lightly, and placed in melted diglycol stearate that has been poured into paper molds. To avoid desiccation of material, only as much of it is encased in the stearate as can be cut on the same day.

The cooling and hardening of the stearate may be speeded up by the placing of paper molds, with contents, in a refrigerator. After the stearate has hard-

ened, the individual pieces of material are cut out; enough of the stearate should be left about each piece so that the encasing medium does not split off from the encased material.

The pieces of stearate with the encased material are then mounted on wooden blocks and sectioned on a sliding microtome. To prevent curling of sections, paper strips moistened in water are used upon the side to be cut (see Varrelman, 1931).

The stearate, which usually enters the large pores in the sections, may be dissolved out by placing the sections in 70 per cent alcohol on a slide-warming plate and leaving them there at least overnight.

The material is now ready for staining and is treated with the following media for the specified lengths of time:

1. Distilled water, $\frac{1}{2}$ to 1 hour.
2. Bismark brown in 1/5,000 dilution in distilled water, 2 hours. (Stock solution of the stain: 1 gram of Bismark brown, 5 grams of phenol, 100 cc of distilled water.)
3. Distilled water, 1 hour.
4. Iodine green in 1/5,000 dilution in distilled water, 2 hours. (Stock solution of the stain: 1 gram of iodine green, 100 cc of 30 per cent alcohol.)
5. Distilled water, 1 hour.
6. Resorcin blue in 1/5,000 dilution in distilled water, overnight. (Stock solution of the stain: 1 gram of resorcin blue, 100 cc of 30 per cent alcohol.)
7. Distilled water, $\frac{1}{2}$ hour or less. (Longer time in water will cause resorcin blue to fade.)

The sections are now ready for mounting on the slides. Each section is brushed gently on both sides with a camel's-hair brush and mounted on slides in dark Karo sirup. (Johansen, 1940, p. 24, recommends this grade of Karo sirup as a mounting medium.) Weights are used on cover glasses for about three days, shell vials with mercury making convenient weights for this purpose.

The schedule just presented may be variously modified, especially with regard to the time limits in the individual stains, and, probably, different schedules will give best results with different materials. The method used in the present study has certain imperfections. A uniform balance between the Bismark brown and the iodine green is difficult to get. The resorcin blue gives a dark precipitate (hence the need for brushing) and sometimes causes a general darkening of the sections. The last washing in distilled water must be carefully watched so as to prevent the fading of resorcin blue.

The permanency of the staining combination is yet to be tested. Some slides that were made in September, 1945, had their original brilliancy a year and a half later; in others the resorcin blue had faded.

The process of mounting in sirup also is somewhat awkward. Since this sirup has a relatively high surface tension, it does not spread well and tends to trap bubbles of air. Its spreading quality may be improved by the use of very clean slides and cover glasses and by the addition of distilled water to the sirup to thin it. However, air bubbles may appear under the cover glass later, when the sirup loses water upon drying. To overcome this difficulty, one should

use the sirup in excess and let it accumulate around the edge of the cover glass. If neater slides are desired, the excess may be cleaned off and the cover glass sealed with Canada balsam around its edge. None of the manipulations just suggested, however, gives a complete insurance against the appearance of air bubbles under the cover glass.

If a quick detection of callose is not essential, Bismark brown and iodine green, without the resorcin blue, are a practical staining combination. The callose remains colorless with this treatment but the connecting strands are deeply stained. Bismark brown and iodine green, followed by mounting in Karo, have been used successfully also on paraffin material (plate 20, A).

Though the iodine green was employed for differentiating the sieve-tube slime, it also added to the quality of the slides by its effect upon the lignified tissues. Plates 6, B, and, particularly, 16, A, illustrate the well-balanced staining of lignified and nonlignified walls that may be attained with these two stains. Bismark brown used without the iodine green leaves the lignified walls too pale (plate 17, A).

Because of their deep staining, the substances usually referred to, in the literature, as tannins detract from the appearance of the black-and-white photographs. Since, however, an excessive denaturing of the material was not desirable in this study, no attempt was made to remove the tannins. These substances were much less conspicuous in sections that were treated only with I_2KI and aniline blue (plates 13 and 15) than in those that were killed in chrome-acetic-formalin or formalin-acetic-alcohol combinations. (Contrast, for example, plates 13 and 15 with plates 8 and 9; the latter illustrate killed sections stained with iodine and aniline blue.) In material that is stained with fast green and safranin, the tannins are usually deep red and can be made less conspicuous in photography by the use of red filters (plates 2 and 3). Such treatment, however, makes the lignified walls less distinct. (Observe the xylem in plates 2, A and B; and 3.)

The combination of the fast green-safranin staining with a red filter in photography is very satisfactory for showing the early stages in the ontogeny of the primary phloem (plate 2).

Most of the photomicrographs were prepared from sections of the Sultanina variety. Plates 8 and 9 were made from the Ohanez variety.

GROSS ANATOMY OF THE AXIS OF THE GRAPEVINE

To orient the reader regarding the spatial relation of the phloem to the other tissues in the stem and root of *Vitis*, the axial parts of the latter are briefly described. As was stated in "Material and Methods," samples were taken from stem parts of different ages. At the end of the primary growth, the internode of a current-year shoot or of a seedling stem (plate 4, B) shows the usual structure of a primary stem of a dicotyledonous vine. The stem is ribbed, with the largest vascular bundles appearing beneath the ribs. Later, as the stem undergoes expansion through secondary growth, the ribs disappear. The cortex is differentiated as collenchyma near the surface of the stem. The collenchyma cells have smaller diameters than the rest of the cortical cells and usually form strands, the largest in the region of the ribs. (In plate 4, B, the collenchyma is rather thin-walled.) One to about four layers of parenchyma

separate the collenchyma from the epidermis. The cortical cells contain chloroplasts, but these are very small in the collenchyma cells. Many cortical cells have tannins. Starch usually accumulates in the cortical plastids. Strikingly large starch grains develop in the starch sheath, that is, in the cortical layer that touches the vascular bundles (plate 4, *A*).

On the inside of the cortex is the vascular region that consists of vascular bundles of various sizes, separated from each other by wide multiseriate parenchymatous rays, the primary rays. Since the bundles have a long parallel course (according to D'Arbaumont, 1881a, the leaf traces of *Vitis* traverse not less than two or three internodes without joining other bundles, and this junction occurs at the nodes), the primary rays also have a long course in longitudinal direction. Thus the primary rays of *Vitis* are both wide and high. The vascular bundles have caps of primary-phloem fibers on their outer sides (plate 4). Beneath the fibers lies the conducting part of the primary phloem. Then follow cambium, xylem, and pith (plate 4, *B*). Toward the end of primary growth, cambium occurs in the bundles and in the rays, that is, it consists of the fascicular and interfascicular portions.

Secondary growth causes two changes in the stem structure: an increase in the amount of vascular tissues through the activity of the vascular cambium and a casting-off of the epidermis, cortex, and part of the primary phloem through the activity of the cork cambium. Plate 5, *B*, shows a transverse section of a shoot at the end of the first year of growth (that is, a "mature cane" of the viticulturist), in which the secondary growth has largely obscured the primary structure. One important feature remains unchanged after the secondary growth occurs—a feature that characterizes the *Vitis* stem as a vine type—namely, the division of the vascular cylinder into strands by the wide parenchyma rays. The interfascicular cambium forms parenchyma cells and thus the primary rays are continued as secondary rays within the secondary vascular cylinder. New rays arise from time to time within the fascicular areas. These rays, of course, are not continuous with the primary rays. For convenience, the secondary rays that are continuous with the primary are here referred to as *secondary rays of the first order*; those that are formed later within the vascular strands themselves, *secondary rays of the second order*.¹ Both kinds of secondary rays are multiseriate, though the rays of the second order are often narrower than those of the first. Vascular elements cross the secondary rays, usually in an oblique direction, and connect the vascular strands with each other. Tracheary elements or fibers form these connections in the xylem region, sieve tubes or fibers in the phloem region (Strasburger, 1891, p. 242 and 250). A phloem connection of this sort is shown in plate 14, *C*. Because of the constant addition of new rays, the subsequently formed parts of the longitudinal vascular system continue to be separated into narrow blocks as they were when secondary growth began. (Compare plates 5, *B*, and 6, *B*.)

Though the subepidermal layer in a *Vitis* stem is as clearly set off from the deeper cortical layers as it is in species that show a subepidermal origin of

¹ To differentiate between the secondary rays that arise from initials in the interfascicular regions and those that are formed within the bundles through conversion of fusiform into ray initials, Barghoorn (1940) used the terms "primary ray" and "secondary ray," respectively. Since both kinds of rays are secondary in origin, Barghoorn's terminology is somewhat ambiguous.

the phellogen (plate 2, A), and though occasional periclinal divisions occur in this layer, the first cork cambium is regularly initiated in the primary phloem and rays of seedling axes and of shoots on several-year-old vines. This deep position of the first phellogen in certain *Vitis* species, including *V. vinifera*, is a well known fact in the literature (Solereider, 1908, p. 224). The cortex and some primary phloem—including the primary-phloem fibers—are cut off by the cork and, after their death, form the dead bark^a on the surface of the shoot. Plate 5, A, shows a transverse section of a seedling stem at the stage

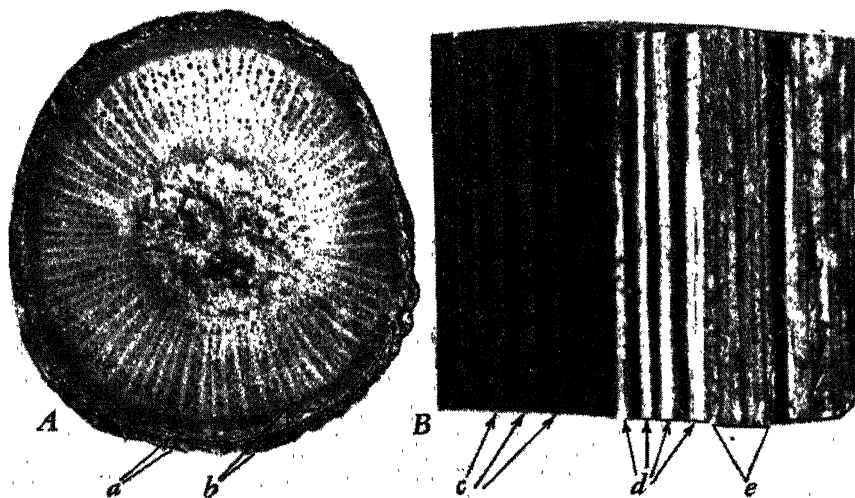


Fig. 1. Transverse (A) and surface (B) views of a cane of the Sultanina variety of the grapevine. Details are: a, dead bark consisting of cortex and some primary phloem with fibers; b, phloem; c, primary-phloem fibers covered by dead cortex (dead bark); d, primary-phloem fibers exposed; e, first cork exposed by removal of primary-phloem fibers. The ridges are the vascular bundles; the grooves are the rays. (Both $\times 5$.)

when cork has been formed and the cortex has dried up (black layer on the surface of the section). The primary-phloem fibers are visible beneath the dead cortex outside the cork.

When cork is formed in a current-year shoot, the latter becomes the "mature cane." A piece of such a cane is shown in sectional and surface views in figure 1. The dead bark (cortex and primary phloem with fibers) is still attached to the cane. Beneath the dead bark appears the phloem (with cork on its outer side). Most of the latter is secondary in origin. Inside of the phloem is the secondary xylem (the cambium is not detectable at this magnification), and next to the pith is the primary xylem. In the surface view, part of the dead bark is intact (to the left in the photograph). Here the primary-phloem fibers form ribs on the surface (fig. 1, B, at c) because the cortex is collapsed and shrunken. At d the primary-phloem fibers have been exposed by peeling off the dead cortex. At e these fibers and the adjacent dead cells have also been removed and the

^a "Bark" is used here in an old-fashioned way to designate tissues outside the vascular cambium; "dead bark" refers only to the tissues that have been cut off by the cork cambium.

cork is exposed. The surface at *e* corresponds to that which forms the outermost layer (cork) in plate 5, *B*. The latter illustration shows that, when the dead bark is removed, the surface of a mature cane is slightly undulate. The surface of the blocks containing the xylem and phloem of the longitudinal system is convex, that of the rays is concave. The undulations appear also in figure 1, *B*, at *e*, where the vascular blocks form the raised strips and the rays occur in the grooves between the strips.

New layers of cork are formed more or less regularly each season around the circumference of the stem so that a "ring bark" is formed (Solereder, 1908, p. 224). Thus every year the vascular cambium adds new phloem, but since the cork cambium cuts off some of the old phloem, the bark remains relatively thin while the wood becomes thicker from year to year. This is shown for a three-year-old branch in plate 6, *B*.

In the present study the root was considered in less detail than the stem. The rootsamples were taken rather infrequently (see "Material and Methods") and consisted of portions of lateral roots that were several years old. No roots in primary state were studied. According to Penzig (1882), the primary-root xylem is diarch or triarch. Secondary growth later produces a solid wood core traversed by many wide rays (plate 6, *A*). The rays are multiseriate and many are wider than those in the stem. (Compare *A* and *B* in plate 6.)

As in most woody roots, the phellogen in the root of *Vitis* arises beneath the endodermis and causes the sloughing off of the cortex. Kroemer (1906) and Szigethi-Gyula (1905) regarded the pericycle, Penzig (1882) the phloem as the place of origin of the first phellogen. Probably both situations occur. In plate 6, *A*, in the lower left-hand part of the section, pericyclic cells appear outside the phloem; this indicates that the cork cambium did not arise in the phloem in this part of the section. In subsequent years cork formation in the root must be rather irregular as compared with the same activity in the stem: the root bark usually appears more uneven and considerably thicker than that of a stem of similar age. (Compare *A* and *B* in plate 6.)

STRUCTURE OF THE PHLOEM

Primary Phloem. The first sieve tubes appear in an internode that has not yet elongated. In transverse sections these sieve tubes are easily distinguished by their somewhat thick, deeply staining walls (plate 2, *A*). The first sieve tube of a given procambial bundle differentiates before the first xylem element of the same bundle (plate 2, *A*, bundle to the left). During the initial stages of phloem development the procambial bundle shows active divisions in the phloem region, with new walls being laid down concentrically about the first sieve tube (plate 2, *A*, bundle to the left). The xylem region appears somewhat less dense than the phloem region; also the cells there are mostly of larger diameters, and early show radial seriation in their arrangement. Thus in the bundle to the right in plate 2, *A*, several radial files of cells appear in the xylem end of the bundle and the first xylem element (*x*) occurs at one end of the median of these files.

Concentric divisions continue in the phloem until many sieve tubes differentiate. The further addition of phloem cells occurs by tangential divisions in that part of the procambium located between the xylem and the phloem. The

bundle in plate 2, *B*, shows the stage of development when the last evidence of concentric divisions appears on the flanks of the phloem, and when the most abundant divisions occur on the inner margin of this tissue. Most of the latter divisions occur in tangential planes but are not restricted to one layer of cells. When the procambium is converted into cambium, at the end of primary growth, the periclinal divisions become restricted to a narrow zone and give rise to cells in both directions, toward the xylem and toward the phloem (plate 3). Thus the transition from the concentric arrangement of cells to the radial seriation occurs gradually. Though a sharp line of demarcation cannot be drawn between the protophloem and metaphloem, the first part of the phloem that arises mainly by concentric cell divisions (plate 2, *A* and *B*) can be conveniently interpreted as protophloem. This part of the primary phloem is most affected by the elongation of the axis and is profoundly modified at the end of this elongation.

The behavior of the sieve tubes is characteristic of protophloem sieve tubes; they function for a short time, are crushed by the adjacent cells, and, finally, are completely obliterated. Plate 2, *B*, shows several stages of this obliteration. Companion cells seem to be lacking, at least in the earliest part of the protophloem. The cells surrounding the sieve tubes in this tissue appear at first as elongated parenchyma cells, some with tannins in their protoplasts. After the sieve tubes are obliterated, the elongated cells differentiate into septate fibers. As is typical of primary-phloem fibers, these cells, before they mature, become many times longer than originally. They grow longer in part by keeping pace with the elongation of the internode, in part by apical intrusive growth. Because of the latter mode of elongation, the ends of the fibers are much pointed and the cells overlap each other. The elongation is accompanied by a lateral expansion. During this active growth, the future fibers appear as very thin-walled, highly vacuolated cells (plate 2, *C*), some of which show dense accumulations of tannins.

After the elongation is completed, the cells lacking tannins develop secondary walls. Some of the tannin-containing cells do not develop thick walls and are more or less crushed by the expanding fibers; others develop into fibers, though often with a delayed formation of secondary walls. Usually, in the end, the mature fibers form solid strands of sclerenchyma tissue with uniformly thickened walls (plates 3 and 4). A fiber cell does not become multinucleate, but in mature state it has thin septa that divide the cell into several compartments, each with a separate nucleus. The protoplasts remain alive until the fibers are cut off by the cork.

As was stated in a preceding part of this paper, cortical cells with large starch grains—the starch sheath—occur on the outside of the sclerenchyma strands (plate 4, *A*). Usually the starch sheath is limited to the vicinity of the fibers, being absent opposite the rays.

The metaphloem that develops centripetally from the protophloem, through tangential divisions in the procambium, consists of sieve tubes, companion cells, and phloem parenchyma. No fibers occur in this tissue. Many of the parenchyma cells contain tannins. Some of the tannin cells are very wide and long (note the large cells in the metaphloem in plates 2, *C*, and 3) and are called, by Strasburger (1891, p. 252), "sac-like parenchyma cells, rich in tannins."

The sieve-tube elements of the primary phloem have transverse or slightly inclined end walls bearing simple sieve plates. The first mature sieve tubes of the protophloem have extremely short elements, the later ones successively longer units. In addition to the sieve plates, the primary sieve tubes, at least those of the metaphloem, have many sieve areas on the longitudinal walls. The usual callus formations occur on these sieve areas and on the sieve plates, and definitive callus is deposited before the sieve tubes are obliterated. Though the walls of the primary sieve tubes are relatively thick, they are less massive than those of the similar elements in many other plant species (Esau, 1939; Schneider, 1945). All primary sieve tubes have slime bodies in their early stages of differentiation and are enucleate at maturity (Esau, 1947).

The first cork cambium is initiated in the primary phloem (metaphloem) just inside of the fibrous strands. The sieve tubes and companion cells are obliterated and the remaining parenchyma cells divide periclinally (plate 3). Such divisions extend also into the interfascicular (ray) areas, but there the cells involved in cork formation lie very near or even next to those initiating the interfascicular cambium (plate 3, *B*). The cork cambium therefore appears to dip in the region of the rays—a feature that is later reflected in the undulate-appearing surface of a mature cane (fig. 1, *B*, at *e*; plate 5, *B*).

The periclinal divisions concerned with the initiation of the cork are repeated in the successive products of the initial cell, so that a radial file of cells is formed, in which the outer and inner faces of the original cell may be recognized. (Compare *A* and *B* in plate 3.) The youngest cells in such a file occur one or two cells outside the inner limit of the file (plate 3, *B*). These young cells could be termed "cork cambium," but their activity is very limited. The fully formed cork consists of only a few layers of cells (plate 15, *D*). Once these are formed, cell divisions cease, the cork matures and without further change serves as a protective layer until the end of the next growth season. Then a new cork layer appears in the secondary phloem (plate 19, *C*). Sometimes, before the new cork arises, the cells immediately beneath the old cork greatly extend radially and divide tangentially (plate 19, *C*, above), but this activity leads to no cork formation since it is interrupted by the development of the deeper-seated cork (plate 19, *C*, below).

The cork cells in the ray region are crowded and compressed and their masses are deeply stained with tannins. These cells give the dark-brown color to the grooves on the surface of a cane (plate 5, *B*).

Whether the cells lying in the path of the originating cork contain starch alone or starch and tannins, they are equally active in the formation of cork (plate 3, *A*) and the starch disappears only gradually in the dividing cells. Before the cork matures, the cells outside of it lose starch, but retain the tannins and crystals. According to Strasburger (1891, p. 251), the tannins later impregnate the cell walls and give the dead bark its brown appearance.

As was mentioned in an earlier section of this paper, the primary-phloem fibers, the cortex, and the epidermis are sloughed off through cork formation (plate 5). Sometimes the first cork arises partly in the secondary phloem, and then some secondary-phloem fibers may be cast off together with the primary. (See also Gard, 1900.) Occasionally, in seedlings, cork initiation was observed in the innermost cortex, that is, outside the primary-phloem fibers.

The metaphloem sieve tubes are obliterated during the first season. The tissue beneath the cork then constitutes the nonconducting (nonfunctioning) phloem, which is composed of intact phloem-parenchyma cells and of crushed sieve tubes and companion cells (plate 15, *D*). Thus a mature cane has no primary phloem in function.

Secondary Phloem. The secondary phloem consists of blocks of tissue of the longitudinal system alternating radially with wide phloem rays, that is, with the blocks of tissue of the transverse system (plate 7, *A*). The longitudinal system consists of two distinct kinds of cell complexes arranged in tangential bands: the bands of fibers and the bands containing sieve tubes, companion cells, and phloem parenchyma. The transverse system is made up of rays of two orders: the rays of the first order—secondary rays that are continuous with the primary rays; and rays of the second order—secondary rays arising in the fascicular areas as the stem increases in circumference. (See also "Gross Anatomy of the Axis of the Grapevine," page 224.) Both kinds of rays are several cells in width, but the rays of the first order exceed those of the second in height. Their cell structures are similar.

The secondary-phloem fibers have moderately thick secondary walls and are septate. (Penzig, 1882, reported that septate and nonseptate fibers occur in *Vitis* in about equal amounts.) In *Hypericum* (Vestal and Vestal, 1940), the septa arise after the secondary thickenings are formed on longitudinal walls and become attached to these thickenings. D'Arbaumont (1881*b*) found, in his studies of different species of *Vitis*, that septa may be formed rather early in the phloem fibers, and that the secondary thickening may or may not continue after the septa are formed. In the present study no exhaustive search for stages in septum development was attempted, but evidence of their late origin was encountered in the xylem. The pits of the secondary-phloem fibers are bordered. They have slitlike openings and an enlarged pit cavity at the pit-closing membrane. (See also Hill, 1908.)

The secondary fibers undergo some apical intrusive growth but remain markedly shorter than the primary-phloem fibers. They occur in tangential bands that are several cells thick and reach from ray to ray. The cells are arranged in radial rows (plates 7, *A*; 8, 9, *B*; 11, *A*; 14, *A* and *B*; 15, *D*; 19, *A*, *C*, and *D*). Sometimes the band is much narrower on one side than on the other; or it does not reach across the entire block of the longitudinal system and is made up partly of fibers and partly of thick-walled parenchyma cells.

The septate fibers are living cells and have a nucleus in each compartment. They remain alive until they are removed by cork formation; and they store starch at certain times of the year. (See also Wilhelm, 1880, and Strasburger, 1891, p. 247-48.)

The tangential bands containing the sieve tubes and the parenchymatous members of the longitudinal system are here referred to as *sieve-tube bands* for convenience. In transverse sections the sieve tubes are usually distinguished by their relatively large size (plate 7, *A*; 8, *A*; 10; 11; 14, *A* and *B*; 16, *A*). Since, however, the ends of most sieve-tube elements are tapering, some of the small cells in a given section may be sieve-tube elements. The smallest cells, which have dense protoplasts, are companion cells. The phloem-parenchyma cells are of intermediate sizes, and some contain tannins (plate 11, *A*).

The sieve-tube elements vary in shape and size (fig. 2, *A-F* and *L-P*). They are derived from fusiform cambial cells (plate 16, *B*), which develop into sieve-tube elements (and their companion cells) either directly or after one or two transverse divisions. The resulting sieve-tube elements are, therefore, either fusiform, with both end walls tapering; or they have transverse walls on

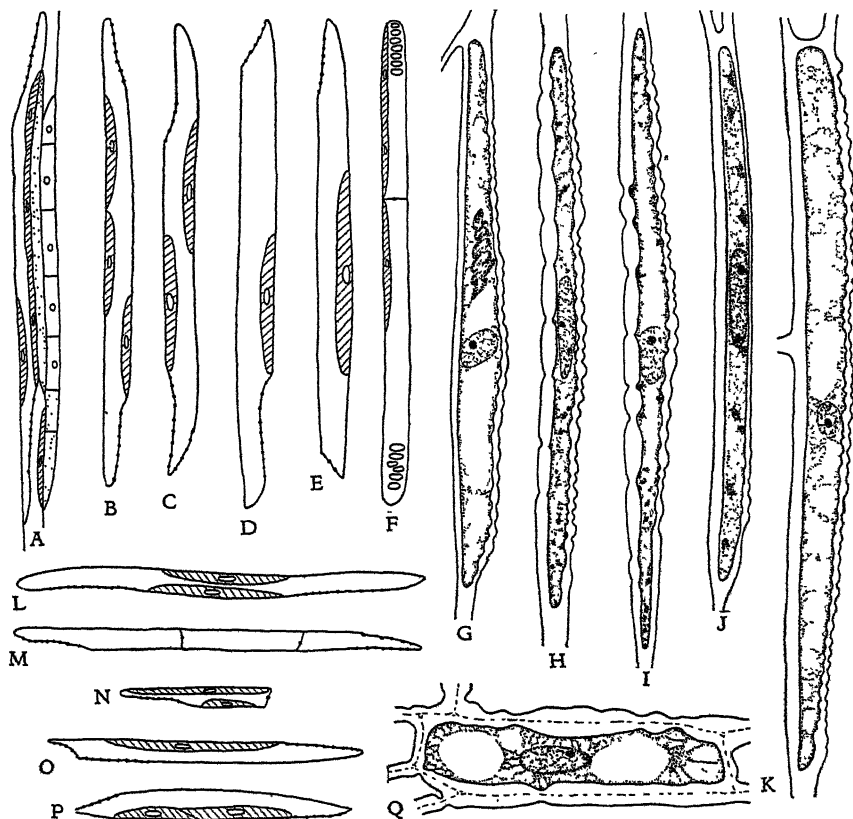


Fig. 2. Longitudinal sections of various phloem cells. *A-F* and *L-P*, Outlines of sieve-tube elements and companion cells (singly hatched). *A* shows also some phloem-parenchyma cells. Among these, the cells with tannins are indicated by stippling and the tannin-free cells by outlines of nuclei. *F* illustrates two sieve-tube elements in radial view with two sieve plates in face view. All the other drawings in this series (*A-E* and *L-P*) are from tangential sections and show the sieve plates (beaded parts of walls) in sectional views. *G-K*, Companion cells. Each of these had the associated sieve-tube element to its right. The youngest companion cell, which contains a slime body (above the nucleus), appears in *G*. *H* and *I* are from fully differentiated, *J* from dormant, and *K* from reactivated phloem. The tannin-free phloem-parenchyma cell in *Q* is from dormant phloem. (*A-F* and *L-P* $\times 100$; *G-I* and *K* $\times 780$; *J* and *Q* $\times 850$.)

one or both ends. The first type predominates in the secondary phloem. Figure 2 gives examples of the two kinds of cells. Each sieve-tube element in figure 2, *A* to *E*, *L*, *O*, and *P*, was derived from one entire fusiform cell. In contrast, one fusiform cell gave rise to two sieve-tube elements in figure 2, *F*, and to three elements in figure 2, *M*. The short cell in figure 2, *N*, is obviously a part

of a fusiform cell. As is characteristic of cambial cells of woody species, the tapering ends of the fusiform initials in *Vitis* are wedge-shaped. The narrow side of the wedge is in view in tangential sections (fig. 2, *A-E*, and *L-P*; plate 7, *B*), the wide side in radial sections (fig. 2, *F*; plate 9, *A*). No apical intrusive growth appears to take place in the formation of sieve-tube elements and the ends of the elements derived from the same part of the cambium occur at nearly the same level (plates 9, *A*; 14, *A* and *B*).

The companion cells are short cells with pointed ends. Each sieve-tube element has one to several (the maximum is five, according to Wilhelm, 1880). These cells in *Vitis* have a historic interest since it was for them that the term "companion cell" was coined (Wilhelm, 1880, p. 4). Being much shorter than the sieve-tube elements with which they are associated, the companion cells do not appear in every transverse section of a given sieve-tube element. Their arrangement varies much in individual elements (fig. 2, *A-F*, and *L-P*). The companion cells of a given sieve-tube element may be either touching, or more or less removed from one another.

Like the primary elements, the secondary sieve-tube members are enucleate when mature and have slime bodies when immature (plate 20, *B*). Slime bodies also occur in the young companion cells, a feature apparently not yet recorded for any other plant (fig. 2, *G*; see also Esau, 1947).

Most of the phloem-parenchyma cells arise from fusiform cells that are divided several times by transverse walls. Therefore, the parenchyma cells appear as relatively short cells arranged in longitudinal files (plates 7, *B*, and 13, *A*). An undivided fusiform cell, or a large part of one, also may differentiate into parenchyma cells (fig. 2, *A*). Many phloem-parenchyma cells contain tannins, which cause the darkening of the protoplasts in fixed sections (plates 7, 8, 9, 11, *A*, and others). At certain times during the year, abundant starch is present in the phloem-parenchyma cells; and this starch may occur together with the tannins.

The sieve tubes are arranged in radial rows (plate 14, *A* and *B*) or are intermingled with phloem-parenchyma cells (plate 8, *A*). The companion cells are usually cut off from a sieve-tube element by a wall that occupies a position intermediate between radial and tangential (plates 11, *A*; 17, *A* and *D*; and 18, *C*). Occasionally a truly radial wall separates the companion cell from the sieve-tube element.

Parenchyma cells commonly occur between the sieve tubes and the rays (plate 7, *B*). According to Wilhelm (1880, p. 5), some of these parenchyma cells are subdivided into small cells containing rhombohedral crystals like those in the marginal ray cells. Occasionally sieve tubes touch the ray cells. Parenchyma cells intervene also between the sieve tubes and the fibers (plate 10, *A*); though sometimes the two latter kinds of elements are in contact with each other (plate 10, *B*, sieve tube to the left, below).

The secondary-phloem rays consist of parenchyma cells. In radial and transverse sections the latter appear as rectangles, slightly elongated radially (plate 7, *A*, to the right), whereas in tangential sections their outlines are rounded (plates 14, *C*, and 16, *B*). If the bark attains considerable thickness, its outer portions undergo dilation—a process that involves only the rays. In this dilation the ray cells stretch tangentially and divide by radial walls. Thus

their original shape is modified: instead of being radially elongated in transverse sections of the bark, the ray cells now are tangentially elongated or isodiametric in such sections. In plate 7, *A*, the ray cells to the right of *a* (in the functioning part of the phloem) have their original shape; to the left of *a* (in the nonfunctioning part of the phloem) they have been subjected to dilation. The ray cells contain tannins or starch (at certain times of the year) or both. The tannins appear in the cells very close to the cambium or are present in the cambial cells themselves (plates 16, *A*, and 18, *B*). Starch occurs also in the cambial ray initials, but only during dormancy. The marginal ray cells commonly become subdivided into smaller cells, each of which develops a rhombohedral crystal (plate 7, *B*, at *b*). The cells containing these crystals do not lose their protoplasts, but the crystal itself becomes imbedded in wall substance that is partly attached to the cell wall (plate 14, *D*; see also Wilhelm, 1880, p. 5). According to Wilhelm (1880), druses may be found occasionally in these cells. When the rest of the ray cells have starch, the crystal-containing cells also form starch. Certain cells within the ray itself develop raphides. These crystals arise in undivided cells near the cambium (plate 18, *B*, at *r*). The protoplasts of the raphide-containing cells do not store starch and eventually become disorganized. The crystals then remain imbedded in a mucilage-like substance. The cells with raphides may be rather large and thick-walled. The occurrence of sieve tubes traversing the rays (plate 14, *C*) has been mentioned in a preceding part of this paper. The elements of these sieve tubes are very short because they arise from the same kind of cells as the rays, but they have companion cells and in their ontogeny pass through the same stages as the sieve tubes of the longitudinal system.

As stated in the beginning of this section, the sieve tubes are usually the largest cells in a given cross section. Certain parts of the phloem, however, contain sieve tubes that appear very small in transverse sections (plate 8, *A*, at *a*). These parts are formed at the end of a growth period. The late-season sieve tubes and the associated cells sharply contrast, by their short radial diameters, with the sieve tubes that are formed earlier in the season and also with those that arise in the beginning of the next growth period. If more than one annual increment of phloem occurs in a given sample of the bark, the small-celled bands serve as a demarcation between the increments. Sometimes the ray cells too are somewhat smaller in the late-season phloem. In plate 7, *A*, and 8, *A*, the small-celled phloem at *a* divides the 1944 and 1945 phloem increments; plate 15, *C*, contrasts the small-celled late phloem with the large-celled early phloem in plate 15, *A*; and plate 16, *A*, shows that though the separation of the annual increments in the phloem (at *a*) is quite distinct, it is less sharp than the demarcation between the annual rings in the xylem (at *b*).

Walls of Sieve Tubes. Although the structure and development of the sieve plates and related structures in *Vitis* have been described in the literature (Wilhelm, 1880; Hill, 1908), they were reconsidered in the present study so as to assure a sound basis for recognizing any existing differences between the newly formed and the reactivated phloem.

Most of the sieve plates are borne on the end walls of the sieve-tube elements (fig. 2, *A-F*, and *L-P*; plates 7, *B*; 9, *A*; and 13, *A*), but some occur on the longitudinal walls. Transverse end walls of short diameters usually bear

simple⁹ sieve plates; others have compound ones. The sieve areas on the end walls (plates 11, *C*, and 12, *C*) lie closer to each other than do those of the sieve plates on the longitudinal walls (plates 11, *B*, and 12, *D*). The sieve plates on the inclined end walls appear in sectional views in the tangential sections of the phloem (plates 7, *B*, and 13, *A*), and in face views in the radial sections of the tissue (plate 9, *A*). The sieve plates of the longitudinal walls are not limited to the radial walls.

Sieve areas less highly differentiated than those of the sieve plates occur on longitudinal walls between sieve-tube elements. In the literature on phloem such sieve areas are often called "sieve fields," in conformity with the terminology introduced by Nägeli (1861). (See also review by Esau, 1939.) Since the two kinds of sieve areas differ only in the degree of specialization and may be connected with each other by transitional structures, designating them by different terms seems unnecessary. Both may be called *sieve areas*, or, synonymously, *sieve fields*. (See also Cheadle and Whitford, 1941.) To specify the reference to the two kinds of structures in this paper, the sieve areas of the sieve plates are called *sieve areas of type A* or *sieve areas A*, and those on the longitudinal walls (excluding the sieve plates on these walls) *sieve areas of type B* or *sieve areas B*. The two types of sieve areas are not so well separated in *Vitis* as in some other plants (Esau, 1939). Nevertheless, the sieve areas A have obviously thicker connecting strands and are associated with larger masses of callus than the sieve areas B. (Compare the sieve areas A in figures 3 and 4 and plates 1, *B*, and 13, *B*, with sieve areas B in figure 4 and plates 1, *C*; 13, *C* and *D*.) The cytoplasm of the sieve tube is more firmly attached to a sieve area A than to one of the other type. Sometimes a series of sieve areas of a sieve plate merge with a series of sieve areas of type B (fig. 4, *G*, and 6, *B*, to the left), or the two are intermingled (fig. 4, *H*).

Hill (1908) stressed the occurrence of "median nodes" (interpreted as swellings of the middle lamella under the influence of ferments) in sieve areas of type B (fig. 4, *D*) and their absence in sieve plates. He concluded, nevertheless, that this was not a fundamental difference and that a sieve area B might become converted into a sieve area A through subsequent enlargement of connecting strands and the concomitant disappearance of the median nodes. In the present study the median nodes of the sieve areas B were observed in some preparations (fig. 4, *D*) and not in others (fig. 4, *E* and *F*).

The sieve areas of the sieve plates develop from the primary pit fields of the cambial walls (fig. 3, *A* and *C*). Occasionally plasmodesmata survive the handling of material and are seen traversing the pit-closing membranes of the future sieve plate (fig. 3, *B*). The number of the plasmodesmata in the pit fields corresponds to the number of connecting strands in the sieve areas of a sieve plate. This relation implies that each plasmodesma becomes one connecting strand of a sieve plate.

As the sieve plate develops, its wall thickens. Though the pit-closing mem-

⁹ The terminology used here with reference to the sieve plates and similar structures agrees with that published by Cheadle and Whitford (1941). According to this terminology, the pores with connecting strands are clustered in the *sieve areas* on the sieve-tube walls. The highly specialized sieve areas (that is, those having relatively large connecting strands) form the *sieve plates*. The sieve plate is *simple* if it consists of one sieve area, *compound* if it is made up of more than one sieve area.

branes increase in thickness, the portions between the pit fields thicken even more, so that in older walls the pit fields become more conspicuous (fig. 3, *D-F*). The thickening of the pit-closing membrane causes the extension of the plasmodesmata (fig. 3, *F*) and is associated with the first appearance of callose (fig. 3, *D* and *E*). Sometimes an original pit field becomes subdivided into smaller ones by an additional thickening of a part of the pit-closing membrane (fig. 3, *F*, second pit field from below).

As has been often described in the literature (Esau, 1939), the callose appears first around each connecting strand on both sides of the pit-closing membrane (fig. 3, *M*). Later the two portions of callose on the two sides of the closing membrane become confluent. The callus is now said to form cylinders, each enclosing a connecting strand (fig. 3, *P*). The cylindrical shape of the calloused portion is soon obscured by the lateral spread of callose near the surface of the wall, but a deeper part of the original wall, between the strands, remains unmodified (fig. 3, *O*). Though sometimes the limits of the calloused areas are very sharp (fig. 3, *O*), they also may appear blurred, and the blue color, imparted to the callose by the aniline blue, may fade out toward the uncalloused portions of the cellulose wall (plate 3, *N*). This observation indicates that callose partly impregnates the cellulose of the sieve-area wall. A substitution of cellulose by callose probably occurs also: when callus disappears in nonfunctioning sieve tubes, the plate exposes pores of as wide diameters as those of the original callus cylinders. Callose does not remain limited to the callus cylinders but is deposited also on the surface of the sieve-area wall, which therefore becomes considerably thickened (fig. 3, *G* and *H*) and eventually the sieve areas cease to be the thin portions of the wall. After the definitive, or dormancy calli develop, the sieve areas are thicker than the intervening portions of the sieve plate (fig. 6, *C*; plate 13, *B*).

While callus appears, the connecting strands of the sieve areas become thicker and their chromaticity increases (fig. 3, *G* and *H*). This change occurs rather abruptly. A comparison of the various parts of the differentiating phloem shows that in the youngest phloem located next to the cambium the connecting strands are hardly visible; outwardly this part of the tissue is succeeded immediately by phloem in which the connecting strands are clearly discernible and are not easily destroyed by various treatments. This change in the appearance of the connecting strands coincides with definite changes in the sieve-tube protoplasts, changes involving the dispersion of slime bodies and the breakdown of nuclei. While the slime bodies and nucleus are intact in a sieve-tube element (plate 20, *B*), the connecting strands are of the nature of plasmodesmata. These strands remain difficult to demonstrate while the slime bodies are spreading out in the initial stages of their dispersion (fig. 6, *A*). Then, after the incorporation of the slime bodies into the vacuolar contents of the sieve tube—and the concomitant breakdown of the nucleus—the connecting strands suddenly become prominent. As has often been recorded in the literature (Esau, 1939), sieve tubes cut after this stage of development have the slime in dense accumulations on the sieve plates, and the connecting strands passing through these plates appear to be made up of slime (plate 12, *A* and *B*; 20, *C*).

The sieve areas of the sieve plate seem to follow two developmental patterns.

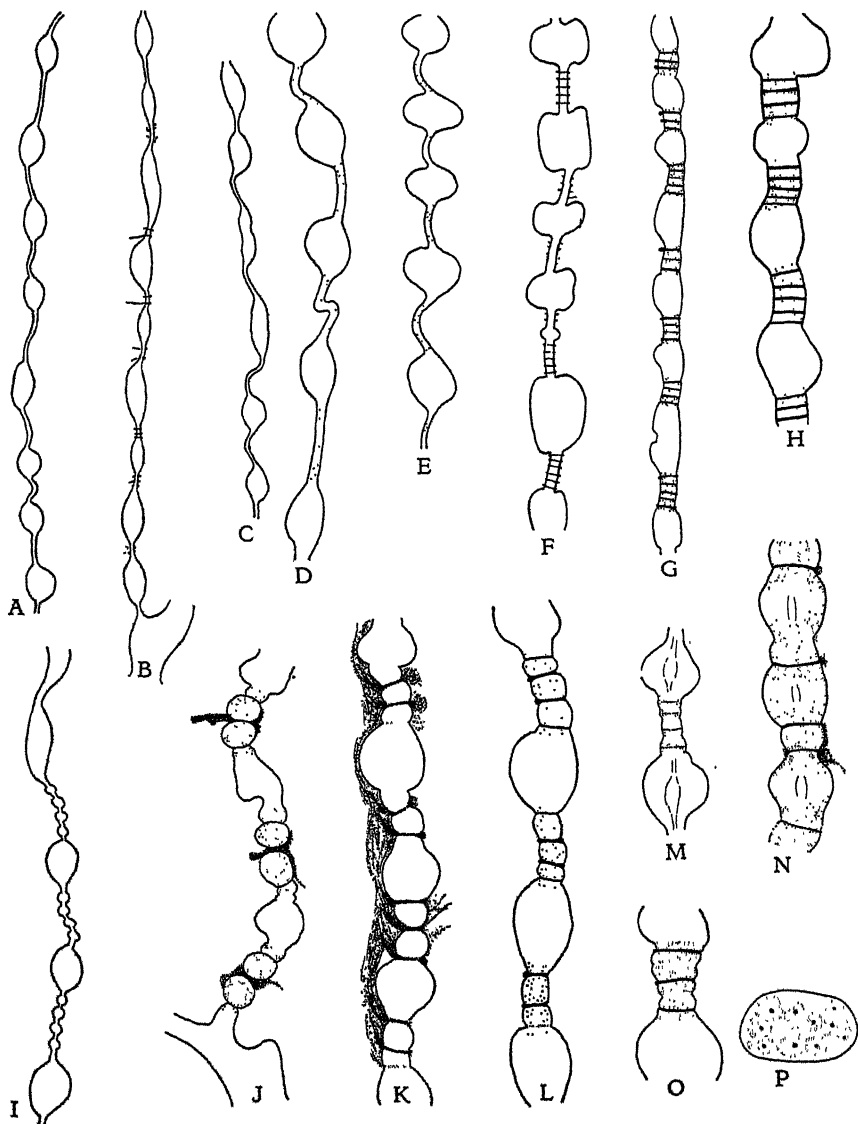


Fig. 3. Longitudinal (A-O) and transverse (P) sections of sieve-tube walls (sieve plates) bearing sieve areas of type A in various stages of differentiation. A-F and I are from immature sieve tubes, the others from mature ones. The calloused areas are stippled in D, E, G, H, J, and L-P. A-C, Young walls with callus-free pit fields. D-F, Sieve plates at the stage when callus is detectable in the pit fields. In B and F plasmodesmata are shown in some pit fields. G and H, Sieve plates (with connecting strands and callus) that were apparently derived from the type of walls shown in A-F. I, Young sieve plate in which the pit fields of the first order are subdivided into pit fields of second order. J-L, Sieve plates that apparently were derived from a type of wall as in I. M-P, Sieve areas showing details of the spatial relation between connecting strands and callus. (A and G $\times 875$; B-F and H-P $\times 1320$.)

In one the sieve area arises through a transformation of a primary pit field in the manner described above; that is, a pit field becomes converted into a sieve area, with the pit-closing membrane thickening uniformly (fig. 3, *C, D, G, and H*), except when the sieve area becomes subdivided into smaller units (fig. 3, *F*). In the other type the primary pit area shows thickenings between every two plasmodesmata, so that, in sectional views, a beaded appearance is noticeable not only in the entire compound sieve plate but in the pit-closing membrane as well. The latter is much more delicately beaded than the former (fig. 3, *I*). The sieve plates derived from such doubly pitted walls have rather widely spaced connecting strands (fig. 3, *J-L*) and these probably attain greater thickness than the strands formed in singly pitted walls. Hill (1908) must have been referring to the same developmental difference when he said that in some sieve plates, as seen in sectional views, one strand occurs in each sieve area, in the other, four to five. The area between two small thickenings (fig. 3, *I*) he evidently termed one sieve area.

The existence of the differences, just mentioned, in the development of the sieve plates justifies combining the sieve areas of the longitudinal walls (sieve areas B) and the sieve areas of the sieve plates (sieve areas A) under one term, *sieve areas*. Though the sieve areas B are less specialized than the sieve areas A, the latter too vary among themselves in the degree of differentiation.

The sieve areas of type B pass through visible stages of development similar to those of sieve areas A. Each sieve area arises from a primary pit field of a cambial wall (fig. 4, *A*). If plasmodesmata are distinguishable in the differentiating walls (fig. 4, *B and C*), their number in the pit areas corresponds to the number of strands in the sieve areas (fig. 4, *D, E, and F*). During its ontogeny, a given sieve area may become subdivided into smaller units by wall thickenings arising later and not attaining the same thickness as the wall parts separating the original primary pit fields (fig. 4, *I*, wall to the right). Though at first each connecting strand has its own callus cylinder (fig. 4, *D*), later the callus formations of one sieve area merge and the same material becomes deposited on the surface of the wall also (fig. 4, *E and F*). In surface views, sieve areas B (fig. 4, *I and J*; plate 13, *C and D*) vary in shape and size much more than sieve areas A (fig. 3, *P*, and plate 12, *C and D*).

Walls of Cells Associated with the Sieve Tubes. Sieve areas of type B occur, but rather infrequently, on walls that separate the sieve-tube element from a phloem-parenchyma cell. As is well known in the phloem literature (Esau, 1939), such walls show sieve areas only on the sieve-tube side (fig. 5, *G-I*), whereas an ordinary pit,¹⁰ at the most, occurs on the parenchyma side. In the sieve-tube wall the connecting strands are associated with callus and are as

¹⁰ According to the Committee on Nomenclature (1933), *pit* designates a recess in a secondary wall only, and *primary pit field* a thin area of the primary wall and intercellular material. However, in the present paper *pit* serves to identify a depression either in a primary or a secondary wall, whereas *primary pit field* is used in reference to walls in the cambium and to the cambium's immediate derivatives. The adoption of a strict distinction between the terms *primary pit field* and *pit* would require an equally accurate differentiation between primary and secondary walls. The precise identification of the nature of walls is often beyond the scope of an anatomical study (or of a classroom exercise). In such a circumstance the investigator (or the teacher) is left without a term for the thin areas in the walls. If *pit* were to be used for these areas without regard to the nature of the wall (as is done in the present paper), *primary pit* and *secondary pit* could be employed whenever a precise distinction between primary and secondary walls is essential.

conspicuous as in the sieve areas B located between sieve-tube elements, whereas in the part of the wall that belongs to the parenchyma cell the connecting strands are like plasmodesmata and are difficult to demonstrate. A sieve area on the sieve-tube side does not necessarily have a clearly defined pit opposing it in the parenchyma-cell wall (fig. 5, *G-I*), though, presumably, plasmodesmata occur in the latter. (See Hill, 1908.) On the other hand,

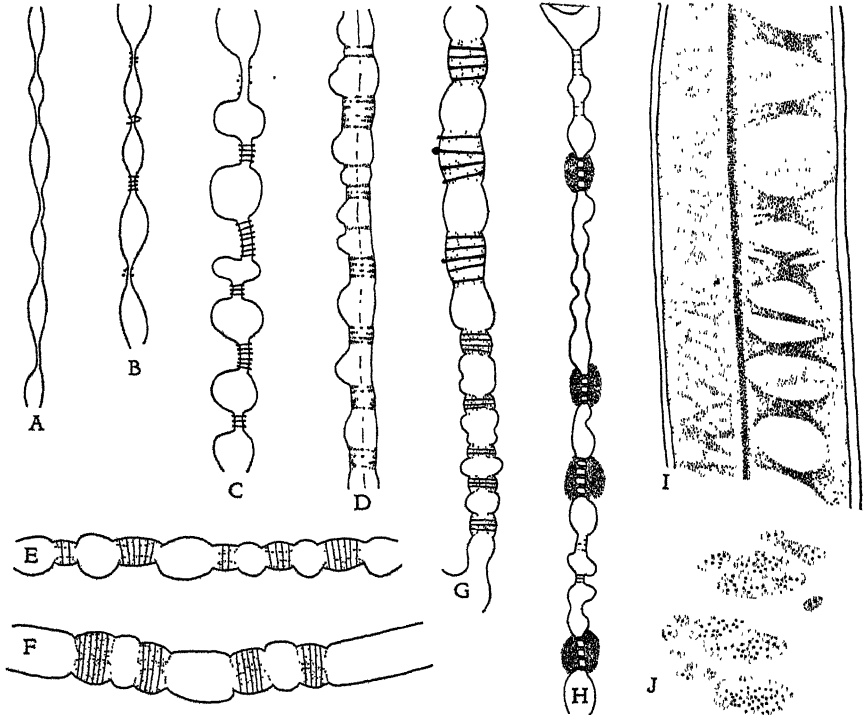


Fig. 4. Sectional (*A-H*) and face (*I* and *J*) views of sieve-tube walls bearing sieve areas of type B. Stippling indicates the calloused parts in *G*, *E*, *F*, and *J*, the slime accumulations in *H*. *A* and *B*, Young walls with pit fields. (Plasmodesmata are shown in *B*.) *C*, Partly differentiated sieve areas with conspicuous plasmodesmata. In *D* the wall is markedly thickened and the connecting strands are associated with callus. (The two are not differentiated from each other in the drawing.) *E* and *F*, Two advanced stages in the development of sieve areas. *G* and *H*, Walls bearing two types of sieve areas, *A* (thick connecting strands) and *B* (thin connecting strands). *I*, Sieve-tube wall with pitting between the sieve tube and a companion cell, to the left, and pitting between two sieve tubes (sieve areas *B*), to the right. The stippled areas are the thick portions of the wall, the unstippled are the depressed portions (pits or sieve areas). *J*, Sieve areas *B* with callus (stippled parts) and connecting strands (large dots in stippled parts). *E* and *J* show sieve areas in similar stages of development. (*A-F* and *J* $\times 1245$; *G-I* $\times 825$.)

parenchyma walls with deep depressions may be associated with perfectly smooth sieve-tube walls (fig. 5, *N*, below); or the latter may show only slight indentations without any connecting strands (fig. 5, *O*, above). If the sieve-tube element and a parenchyma cell are connected by a sieve area-pit complex, callus accumulates only on the sieve-tube side (fig. 5, *I*). These unilateral deposits of callose are particularly striking in dormant phloem and they appear to be formed even in wall parts where no pits or sieve areas can be ex-

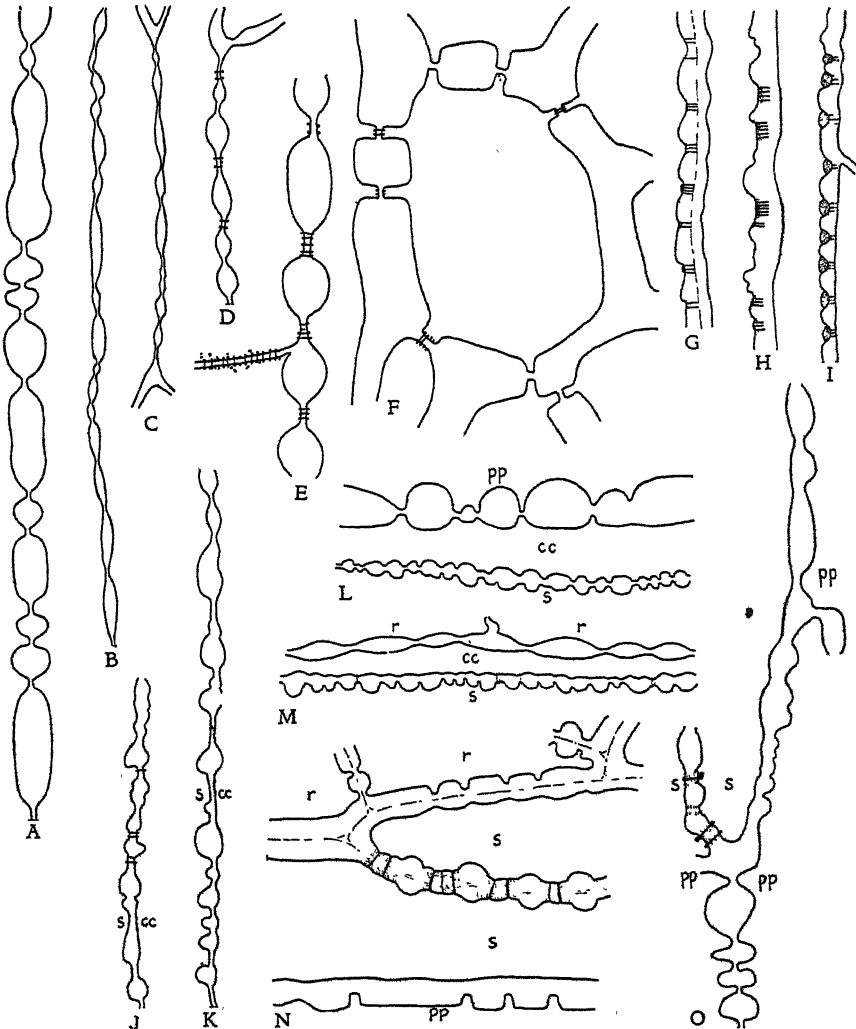


Fig. 5. Sectional views of various cambium and phloem walls. Cambial walls during dormancy (*A*) and during growth (*B* and *C*). Younger (*D*) and older (*E*) phloem-parenchyma walls with plasmodesmata in the pits. *F*, Ray cell with plasmodesmata in some pits. *G-I*, Walls between sieve tubes and phloem-parenchyma cells from tangential (*G* and *I*) and radial (*H*) sections. The connecting strands in *G* and *H*, and the callus in *I* are visible only on the sieve-tube side. *J* and *K*, Walls between sieve tubes (*s*) and companion cells (*cc*), with plasmodesmata in some pits. *L-O*, Walls between different cells whose identity is indicated by the small letters. Details are: *cc*, companion cell; *pp*, phloem-parenchyma cell; *r*, ray cell; *s*, sieve-tube element. (*A-H* and *J-L* $\times 1175$; *I* and *M-O* $\times 780$.)

pected; namely, opposite the transverse walls separating phloem-parenchyma cells from each other (plate 1, *D*). Where a sieve tube occasionally touches a ray cell, the latter may show rather deep depressions in its wall, but the associated sieve-tube wall has, at the most, only shallow indentations. In this study, no connecting strands were noted in walls between the sieve tubes and rays.

The wall between a sieve-tube element and a companion cell is relatively thin but is profusely pitted (figs. 2, *G-K*, walls to the right; 5 *J-M*). Plasmodesmata occasionally remain preserved in these pits when fresh sections treated with iodine and aniline blue are used (fig. 5, *J*). According to Hill (1908), the connections between the sieve tubes and companion cells are similar to those between the sieve tubes and phloem-parenchyma cells. Because of the thinness of the wall between the companion cell and the sieve tube, this point is difficult to ascertain. After a long search, callus was found on the sieve-tube side of such a wall, but only in dormant phloem. In face view, the pits on this wall resemble the sieve areas *B*, but are smaller (fig. 4, *I*, cell to the left). The wall between a companion cell and a phloem-parenchyma cell is usually prominently pitted (fig. 2, *H* and *I*, wall to the left; 5, *L*). The wall between a companion and a ray cell is either shallowly pitted (fig. 5, *M*) or is smooth.

The longitudinal walls separating phloem-parenchyma cells from each other are rather thick and have deep pits (fig. 5, *O*, below). The transverse walls are generally thin and without pits. If plasmodesmata are evident in the sections, they are confined, in the longitudinal walls, to the pit-closing membranes; but in the transverse walls are scattered throughout (fig. 5, *D* and *E*). The ray-cell walls, as seen in tangential sections (fig. 5, *F*), are prominently pitted where these cells touch each other or the phloem-parenchyma cells. The plasmodesmata are confined to the pit-closing membranes.

If the presence of sieve areas and pits signifies the existence of interconnections between protoplasts, then one might summarize the relation of the phloem cells to each other as follows. The sieve-tube elements are intimately connected with each other by sieve areas of different degrees of specialization; they are connected with the companion cells by pits that are probably modified into sieve areas on the sieve-tube side. Few or no connections exist between sieve tubes and the other parenchymatous members of the phloem (phloem-parenchyma and ray cells). Companion cells, on the other hand, are connected with parenchyma and ray cells by pits. The relation of the companion cells to each other may be disregarded because they rarely touch one another. The common walls of the fibers are pitted, and pits occur also between the fibers and the phloem-parenchyma cells that are in contact with the marginal fibers.

The cambial cells and their immediate derivatives that give rise to the various phloem cells show similar unevenly thickened walls (figs. 3, *A-C*; 4, *A*; 5, *B* and *C*). In other words, they all show primary pit fields. These structures then undergo various modifications, depending on the type of cell in which they occur.

Since each wall is actually composed of two parts—one from each of the two cells that occur side by side—the pit fields, too, are double structures: they are pit-field pairs. In such a pair the two members develop differently when a sieve area differentiates on the sieve-tube side and a pit on the phloem-parenchyma side. The pits in parenchyma walls visibly differ from the primary pit fields of the active cambium by the greater thickness of their wall parts. This difference is obviously not fundamental, since, during dormancy, cambial walls may be as thick and have as deep depressions as the parenchyma cells in the fully developed phloem (fig. 5, *A*).

As far as could be judged from this study, all phloem cells, except the fibers, have primary walls. The sieve tubes show no transitory thickening (*nacré*) comparable to that of the sieve tubes of many other plants (Esau, 1939).

Characteristics of the Functioning Phloem. The terms *functioning phloem* and *nonfunctioning* (or *functionless*) *phloem* are used here to differentiate between the tissues containing functioning sieve tubes and nonfunctioning sieve tubes, respectively. According to this terminology, the functioning phloem is concerned with the principal activity of this tissue (food conduction) as well as with certain other functions, whereas the functionless phloem has ceased to conduct but may still be concerned with starch storage, protection, and other functions. Thus the differences between functioning and nonfunctioning phloem are largely determined by the condition of the sieve tubes and their companion cells. The characteristics, which the sieve tubes assume at the end of their differentiation and which they retain in a more or less constant state during growth, flowering, and fruit ripening of the grapevine, are here interpreted as the characteristics of functioning or mature sieve tubes. Since these features have been considered from various viewpoints earlier in the paper, they may be briefly summarized here. The general appearance of the functioning phloem is shown in plates 7, *A*, to the right of *a*; 7, *B*; 8, *A*, to the right of *a*; 9, *A*, and 13, *A*.

In a mature sieve tube the enucleate protoplast contains a vacuole in which the slime is dispersed. In cut material this slime accumulates in various amounts on the sieve plate and is continuous through the pores. Because the slime stains well, its presence in the sieve-plate pores makes the connecting strands very conspicuous in sieve tubes of the active phloem (figs. 3, *G*, *H*, *J*, and *L*; 6, *B*; plates 12, *A* and *B*; 20, *C*).

The amount of callose on the sieve areas varies within certain limits. Generally, each connecting strand is imbedded in a callose cylinder, and some callose covers the surface of the sieve area as well. The callose varies in thickness on sieve areas *B* also, but, in general, the connecting strands of these areas are most prominent during the functioning stage. The longitudinal walls of the sieve tubes are thick, but not noticeably thicker than those of the parenchyma cells (plate 8, *A*). As was mentioned previously, the peculiar thick wall, which occurs in the differentiating and recently matured sieve tubes of many plants (Esau, 1939), is not evident in the grapevine sieve tubes.

Plastids containing starch, which stains red with iodine, are present in the protoplast (fig. 6, *B*; plate 12, *A*). Since the starch grains often shown Brownian movement, their occurrence in the vacuoles—at least in cut material—may be assumed. (See also Esau, 1939.)

The mature companion cells are nucleate cells without starch. As was described in an earlier paper (Esau, 1947), the companion cell has at first a thin protoplast in which a slime body differentiates (fig. 2, *G*). The latter disperses, leaving somewhat deeply staining particles in the cytoplasm (fig. 2, *H* and *I*). Though the mature protoplast is vacuolate, it appears denser than that of a younger cell, probably because of the presence of slime.

The phloem parenchyma varies little in active and inactive phloem. The principal variation is in the amount of starch, which is at a minimum during the most active growth of the plant. (See also p. 267–68.)

Phloem during Dormancy. As was stated in the review of literature, *Vitis* phloem functions for at least two seasons, but the period of activity is interrupted by one of dormancy during the winter. The most outstanding feature of the dormant phloem is the presence of heavy masses of callose on the sieve plates and sieve areas B (fig. 6, *C*; plates 13, *B* and *C*; 14, *A*, *C*, and *E*; 15, *A-C*). This callus is referred to in the literature as *provisional* (Esau, 1939); *dormancy callus* seems an appropriate term also.

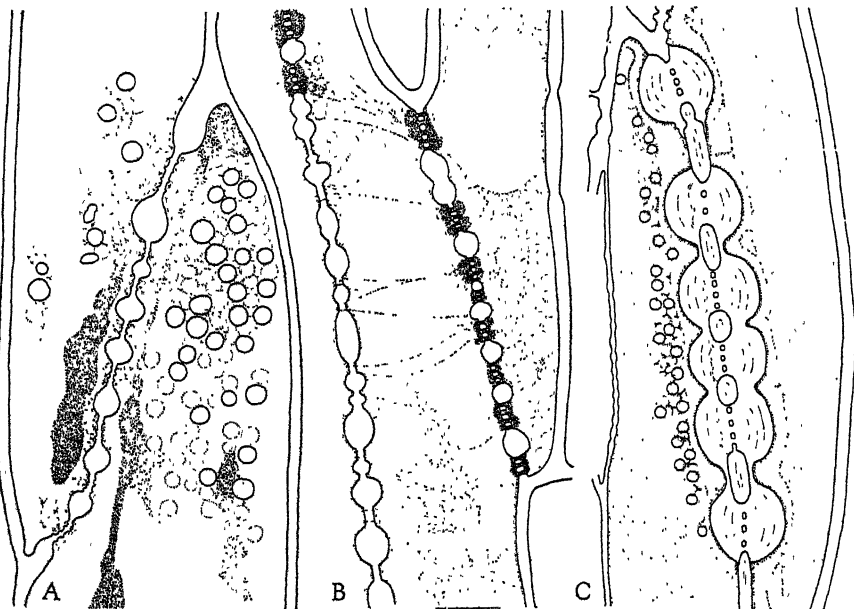


Fig. 6. Longitudinal sections of parts of sieve tubes in different states of activity. An inclined compound sieve plate occurs in the middle of each drawing. *A*, Sieve plate and adjacent structures from an immature sieve tube. The sieve plate shows pitting of two orders like that in figure 3, *I*, but no connecting strands. The densely stippled areas indicate the slime. Nuclei were still present in the two sieve-tube elements whose ends appear in *A*. *B*, Sieve plate and adjacent structures from a completely reactivated sieve tube. The dark material that fills the sieve areas and is continuous through their pores is slime. The cytoplasm is lightly stippled. *C*, Heavily calloused sieve plate from a dormant sieve tube. Slime (stippled) appears next to the sieve plate but is not attached to it. The circles in all three drawings indicate starch gains. (All $\times 733$.)

The dormancy callus is very thick in the large sieve tubes of the early-season phloem (plate 15, *A*) and is moderately thick in the narrow elements of the late phloem (plate 15, *C*). Using European material, Lecomte (1889) found that the definitive callus was thicker than the transitory. In the California material of the present study, the dormancy callus was generally much thicker than the definitive (compare plates 15, *A*, and 19, *B*). Very often the masses of dormancy callus of the individual sieve areas fuse and bury the thick cellulose portions that separate the areas from each other (fig. 6, *C*, and plate 20, *D*). The callus shows layering parallel to the surface of the sieve area plate 15, *B*), and sometimes it peels off, in the cut material, along the lines separating the layers. Occasionally most of the callus peels off, leaving the

plate just slightly thicker than it was during the active period. The cellulose base of the sieve area usually shines through the callus. It is whitish yellow in color and, in material stained with aniline blue, is traversed by blue streaks indicating the callus cylinders in the pores (plate 15, *A*). In surface view, a sieve area appears blue throughout, but the callus cylinders are of a deeper blue and each has a dot in the center.

Using various techniques, Wilhelm (1880) was unable to recognize connecting strands through the callus in dormant sieve tubes, while Hill's (1908, p. 276) report on this matter was rather vague. Hill said that the "pores of the old strings were seen only with difficulty" in material collected early in April and, supposedly, this material was similar to that sampled during dormancy in February and March. Mühldorf (1937) reported having demonstrated cytoplasmic connecting strands in winter callus in *Vitis* by subjecting sections to autolysis in a warm oven and a subsequent test for presence of oxydase. The reagents described in "Material and Methods" in the present study revealed no connecting strands in the provisional callus during the height of dormancy. A technique for demonstrating plasmodesmata (Crafts, 1931) was tried also and gave uncertain results: in a sample collected on January 4, 1946, some callus masses showed faint lines crossing them, others did not. Perhaps thin cytoplasmic strands are present in the callus, but the slime of the dormant sieve tubes is usually completely disconnected from the callus masses (fig. 6, *C*). An exception to this behavior of slime is illustrated by plate 20, *D*: the tough stringy slime is continuous between two elements through one large opening in the otherwise heavily calloused sieve plate. Such connections were found in several samples of dormant phloem. Similar unusually thick connecting strands, one to a sieve plate, were recorded by Nägeli (1861, plate II, fig. 26) in *Cucurbita* sieve plates covered with definitive callus.

According to Lecomte (1889) and Strasburger (1891), the starch and slime almost disappear from the sieve tubes before dormancy. Wilhelm (1880), however, found no differences in the sieve-tube contents during the summer and winter. In the California material used in the present study, the starch content did not diminish in the dormant sieve tubes, and the slime showed variations in amount not unlike those encountered in the active sieve tubes. Sometimes the slime was almost absent; or it was spread out thinly and uniformly as jellylike, lightly staining mass through an entire element or part of it (fig. 6, *C*); or it was shrunk into a tough, dense, partly coiled strand, very distinct from the parietal cytoplasm of the sieve-tube element. The principal structural difference between the dormant and the active sieve tubes was the previously indicated usual lack of slime continuity through the sieve areas in the inactive state.

In the dormant phloem the sieve-tube starch occurs in the parietal cytoplasm outside the slime. In figure 6, *C*, the starch grains were drawn in the same plane as the slime (just to show that starch was abundant in dormant sieve tubes), but in reality they came into view at a different focus than the slime. They obviously were confined to the peripheral portion of the protoplast.

The companion cells, according to Strasburger (1891), have thin protoplasts and are partly collapsed during dormancy. In the material of the present study, the density of the dormant companion-cell contents was equal to or

even greater than that of companion-cell contents in active phloem. The protoplasts of dormant companion cells often showed no conspicuous vacuoles and the dense substance—probably the remainder of the slime bodies—formed deeply staining flakes, somewhat larger than similar flakes in the active tissue (fig. 2, *J*). In contrast, the phloem-parenchyma cells, if not filled with starch, commonly appeared much vacuolated (fig. 2, *Q*). Most phloem-parenchyma cells, all ray cells, and the fibers contain much starch during dormancy.

Phloem during Reactivation. At the break of dormancy the connecting strands again become clearly evident in the callus covering the sieve areas. The slime that during dormancy is not connected to the callus, now appears to adhere closely to it. If no slime was evident before, it now becomes conspicuous, and if its chromaticity was low, the latter is restored in its intensity. At first very thin connecting strands traverse the callus masses—a stage that is not readily detected. Later the connecting strands are much thicker, stain deeply, and are obviously related to the slime accumulations next to the callus. They do not at once appear traversing the whole callus mass, but, as Hill (1908, p. 276) has graphically expressed it, the slime seems “to force its way through the old pores in the callus masses” (plate 20, *A*). Often a strand single at one end of the callus mass is branched in its interior (plates 10, *B*, below; 20, *A*); or a strand is thick at one side of the callus mass and very thin at the other. These observations suggest that at first only thin cytoplasmic strands traverse the callus (perhaps these strands are present during dormancy but are not very chromatic); then a dissolution of the callus along these strands widens the passages and permits the entry of slime into the latter.

As the reactivation progresses, the callus is gradually dissolved. Its surface, as seen in sections, appears festooned (plate 10, *B*, below); then the callus diminishes in thickness until it becomes as thin as it was when the sieve tube first differentiated from cambial derivatives.

The Bismark brown-iodine green-resorcin blue staining combination is very useful for the study of reactivation. The connecting strands are so clearly differentiated from the callose (plates 1, *A*; 10 and 11) that the recognition of the early stages of reactivation in survey work is much facilitated.

Reactivation begins near the cambium, and since the callus masses there are only moderately thick, the youngest sieve tubes quickly assume the appearance of fully active elements. The sieve tubes located farther from the cambium are reactivated somewhat later and more slowly. Plates 10, *A*, and 11, *A*, may serve to illustrate the progressive reactivation of the phloem from the cambium outward. In the section in plate 11, *A*, which occurred next to the cambium, the connecting strands were present in all callus masses and were most prominent closest to the cambium (to the right in the photograph). In the section in plate 10, *A*, which was located near the periphery of the cane, one callus mass (below in the photograph) was completely traversed by connecting strands, the other (above in the photograph) showed only a few beginnings of such strands. (See also plate 1, *A*.) Plate 11, *B* and *C*, shows longitudinal sections of sieve plates from partly reactivated sieve tubes taken in a part of the phloem similar to the section in plate 11, *A*.

The reactivating phloem can be clearly distinguished from the dormant, even in low-power views, by the relative thinness of the callus masses and by

the conspicuousness of the slime. Plate 14, *A* and *B*, illustrates these points of difference. In the dormant 1944 phloem in plate 14, *A*, heavy deposits of callose occur on all sieve areas that are evident in the section, but no slime is visible. The reactivating 1944 phloem in plate 14, *B*, shows thin callus masses and dense accumulations of slime connected to the sieve areas. A comparison of sections from dormant phloem in plates 13, *B*, and 15, *A* to *C*, with those from the partly reactivated tissue in plates 10 and 11 also give a good notion of the morphological distinction between active and inactive phloem.

The companion cells too show some change during reactivation: their protoplasts again become somewhat thinner in appearance, sometimes even thinner than in the preceding season (fig. 2, *K*). The phloem parenchyma, ray cells, and fibers show a gradual depletion of starch.

Certain characteristics of the dormant phloem seem to be, at least in part, indications of reduced amounts of water in the tissue. Such characteristics are: the rather uniform jellylike appearance of sieve-tube contents (fig. 6, *C*) or the much contracted condition of the slime (plate 20, *D*); the comparatively dense protoplasts of the companion cells (fig. 5, *J*); and the accumulation of starch in the parenchyma and fibers.

Characteristics of the Functionless Phloem. Since the old phloem is periodically sloughed off in the grapevine, comparatively small amounts of nonfunctioning phloem accumulate in the bark. More functionless phloem accumulates in some parts of the plant and in some varieties than in others. Certain environmental conditions and presence of a disease also may be expected to affect the amount of nonfunctioning tissue in the bark.

Normally the grape phloem ceases to function as a conducting tissue in the second year of its existence. It may or it may not become separated from the axis at the same time. Accordingly, the characteristics assumed by the functionless phloem vary in relation to the length of time that such phloem remains attached to the plant in unity with living tissues. If the phloem is cast off soon after its sieve tubes cease to function, all its cells die. If the nonconducting phloem remains attached to the axis, its parenchymatous members (excluding the companion cells) and the fibers remain alive and continue to be concerned with starch metabolism.

As was pointed out previously, phloem ceases to function as a conducting tissue when its sieve tubes become disorganized. The disorganization may occur in three different ways and the variations in this phenomenon are related, at least partially, to the length of time the functionless phloem remains connected with the stem.

The first type of disorganization involves a crushing of the sieve tubes and companion cells by the adjacent parenchyma cells. The crushed cells seem like extremely thick walls, but closer examination reveals the slitlike remnants of the obliterated lumina (plates 10, *A*, and 18, *A*). Before the sieve tubes are crushed, their contents may become transformed into a homogeneous mass of material that stains very lightly with aniline blue. Since this substance appears to be a remainder of sieve-tube contents (perhaps of the slime), it is here referred to as *residual material*. No discrete structures, such as plastids or starch, are present in it. Some sieve-tube elements are without the residual material and appear empty, except for the shrunken fragments of the dis-

integrated protoplasts. The sieve plates lack callose—if definitive callus is formed in advance of the obliteration, it disappears before the sieve tubes are completely crushed—and, of course, have no connecting strands.

The method of sieve-tube obliteration just described is characteristic of primary phloem (plate 2) and of that part of secondary phloem that occurs beneath the cork, particularly in the canes (plate 15, *D*).

The second type of phloem disorganization involves filling of the sieve tubes with tyloses—a phenomenon that has been recorded in the old literature on phloem (Janczewski, 1881; Strasburger, 1891). The tyloses are outgrowths of phloem-parenchyma cells (fig. 7, *A*). Before these outgrowths are formed, the protoplasts of the sieve tubes and companion cells die. Residual material may or may not be present in the sieve tubes but starch grains disappear. Some sieve plates show definitive callus, others are without any callus. If this material is present, it stains very lightly with aniline or resorcin blue.

Since the tyloses develop in great numbers in the phloem, and since pits (and sieve areas) are not too common on the walls between the sieve tubes and parenchyma cells, phloem tyloses may not necessarily arise from the enlargement of pit-closing membranes as do those of the xylem. In fact, the tyloses in the sieve tubes have wide bases where they are connected with the initiating cell, whereas similar connections in the xylem are as narrow as the pits. Probably any part, or at least any thin part (fig. 5, *O*), of the common wall between a sieve tube and a phloem-parenchyma cell, is capable of extending into a tylose. If the entire common wall extends, the sieve tube is simply crushed; if parts of such walls extend, tyloses arise. Repeated observations show that tylose formation and partial or entire crushing of the sieve tubes are combined in the same part of the tissue.

Tyloses contain nuclei, starch (fig. 7, *A*; plate 8, *C*), and sometimes tannin. If the tissue remains alive for a relatively long time, tyloses develop thick, profusely pitted walls. If the sieve tube contains residual material, the latter is pushed aside by the tylose (fig. 7, *A*; plate 8, *C*). Tyloses come in contact with the sieve plates that are with or without callus. They may push part way through a sieve plate by stretching the callus-free wall of a sieve area. The typical aspect of phloem with abundant tyloses is illustrated in plates 8, *B* and 9, *B*. In the transverse section in plate 8, *B*, the sieve tubes with tyloses (*t*) appear as wide-open cells, those without tyloses (*s*) as partly crushed structures containing residual material. In the radial section in plate 9, *B*, no long cells with wide lumina—the sieve-tube elements (as in plate 9, *A*)—can be recognized, because these elements are subdivided into small compartments by the tyloses. The companion-cell protoplasts die in the phloem containing tyloses. Occasionally the latter are formed in the companion cells also.

The occlusion of the sieve tubes by tyloses commonly occurs in large branches (arms), main trunk, and roots, where more than one annual increment of phloem may accumulate; but it also occurs in canes.

In the third type of disorganization of the phloem, the sieve tubes are emptied and are rendered functionless without being crushed or closed by tyloses. Remnants of protoplasts occur in such sieve tubes for a time, but residual material is rare. Definitive callus may or may not be deposited on the sieve areas. After it is formed, the connecting strands disappear because of

the death of protoplasts (plate 19, *B*). The protoplasts of the companion cells die as the associated sieve tubes cease to function.

The phenomena just described occur in the phloem that functions till the end of the season and then is cut off by the cork in the normal process of bark sloughing. After the sieve tubes become functionless in such phloem (plate 7, *A*, to the left of *a*), the latter differs much less from the functioning phloem (plate 7, *A*, to the right of *a*), than the tissue containing tyloses (plates 8 and 9). But soon certain phloem-parenchyma cells enlarge radially, divide tangentially (plate 19, *A*), and form several layers of cork (plate 19, *C*, at *c*). While this growth occurs, the cells in the nonfunctioning part of the phloem begin to collapse. All living cells die but certain inclusions, such as tannins and crystals, are not removed from them. The tissue that is cut off becomes the dead bark. Quite commonly the empty sieve tubes are very conspicuous in the dead bark because their large lumina stand out among those of the other more thoroughly collapsed cells (plate 19, *D*). Definitive callus is present occasionally in the dead phloem. The fibers lose their protoplasts also but do not change their shape because of the rigid secondary walls.

SEASONAL CHANGES IN THE PHLOEM

Histology of Cambial Reactivation. A consideration of phloem activity would be incomplete if it were not related to the activity of the cambium from which new phloem arises. As the review of literature in the present paper shows, many workers have studied cambial reactivation in woody dicotyledons and all have generally concluded that springtime cambial divisions are initiated beneath the buds and then spread downward to the main branches, trunk, and roots. *Vitis* also shows this common pattern of cambial reactivation and it exhibits the usual histologic details in the resumption of growth by the vascular meristem.

During dormancy the latest xylem elements of the preceding growth season are easily recognized by their thick, lignified walls. The cambium and the nearest phloem cells, however, are indistinguishable on the basis of wall thickness: both have thick, nonlignified walls (plates 15, *C*, and 17, *A*). Wall thickness alone, therefore, does not indicate the layer (or layers) that should be regarded as cambium proper, nor the initial layer. Since, however, sieve tubes occur sometimes in the second layer from the xylem (fig. 7, *B* and *D*), only one layer of cells could be called "cambium," namely, the layer between the xylem and the nearest sieve tubes. The occurrence of tannin cells in the second row from the xylem (plate 17, *A*) suggests the same arrangement, though the presence of tannins does not always prove that the cell is outside the meristem; in the interfascicular area some ray initials contain tannins (plate 18, *B*). The ray initials also store starch during dormancy—a feature that is not characteristic of the initials in the fascicular areas. (Lodewick, 1928, also observed starch in the ray initials of dicotyledonous trees.) The cambial cell in the fascicular area always lies next to the xylem and sometimes only one phloem-parenchyma cell separates it from the phloem fibers (fig. 7, *C*).

The resumption of cambial activity is first expressed in an enlargement of the cambial cells parallel to the radius of the stem (plate 17, *B*, two cells in median part of photograph). This phase is soon followed by that of tangential

divisions (plate 17, *B*). During the initial enlargement of the cambial cells, their radial walls stretch and become thin, particularly in the region where the new tangential wall is to be attached. In sections, this thin part of the wall easily breaks (plate 17, *C*). After the cambium has been active for a while, this localized thinning down of the wall is not conspicuous because the walls are rather thin throughout. Furthermore, the tangential divisions follow one another so rapidly that the radial extension of the cell, which precedes a division, becomes very limited. The cambium and its immediate derivatives now

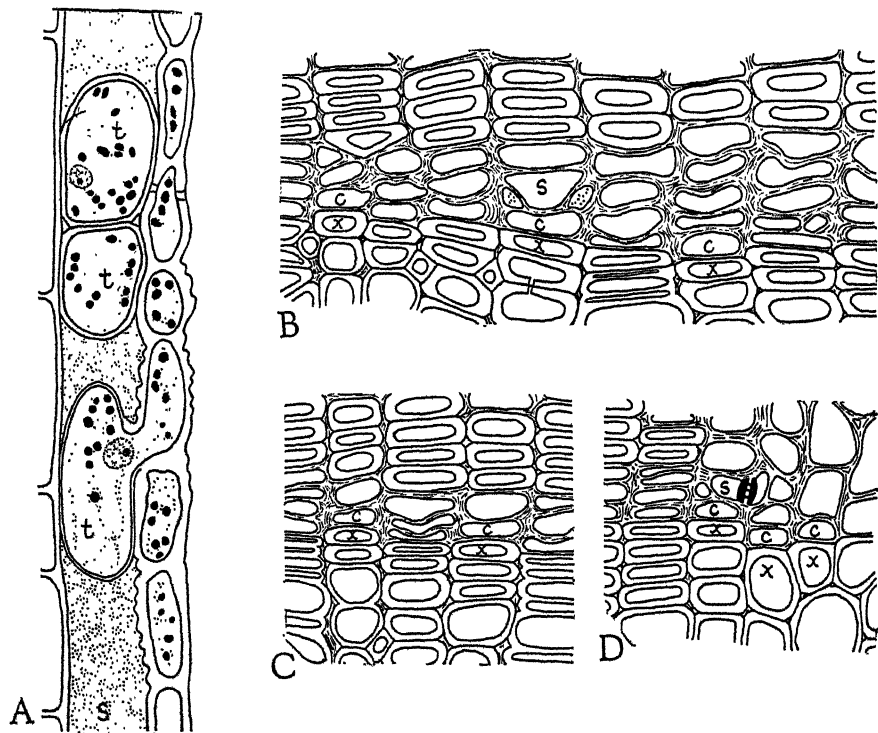


Fig. 7. *A*, Longitudinal section of part of a sieve-tube element (*s*) containing tyloses (*t*) and residual material (uniformly stippled). To the right of the sieve-tube element are phloem-parenchyma cells, one of which is continuous with a tylose. The black spots in the parenchyma cells and in the tyloses represent starch grains. *B-D*, Transverse sections of dormant cambium and adjacent cells from a cane. Details are: *c*, cambium; *s*, sieve tube; *t*, tylose; *x*, xylem. (*A* $\times 360$; *B-D* $\times 457$.)

give the common picture of an active cambial region: they form radial files of cells, which are flattened perpendicularly to their tangential diameters (plate 18, *C*). In this complex the radial walls of the cells engaged in the most active divisions are weak, as are those of the cells that enlarge in the first phase of cambial reactivation, and they too break readily in the sections (plate 17, *D*). The obvious result of this weakness of the radial walls, during the earlier and later stages of cambial activity, is the slipping of the stem bark that is easily induced by mechanical means when spring growth begins.

While, during dormancy, the cambium can be identified as a single layer

next to the xylem, the recognition of the initial layer is rather problematical when its undifferentiated products accumulate. In plate 18, *C*, the initial layer appears to be at *c* since this region shows some recently formed tangential walls. Where such walls do not occur, however, the cambial cells and their immediate derivatives are alike. Judging by plates 17, *D*, and 18, *C*, in the fascicular areas the presence of tannins may be taken as evidence that tissue differentiation has begun: no tannin cells occur in the initial layer and its immediate derivatives. The sieve tubes also begin to differentiate close to the initial layer (plates 17, *D*, and 18, *C*, at *s*), so that, in general, only a relatively narrow band of cells having no obvious characteristics of phloem or xylem elements occurs in the cambial region. The phloem fibers (above the young sieve tubes in plate 18, *C*) resemble cambial cells until their walls begin to thicken. Plate 16, *A*, illustrates particularly well the similarity between the region where cambial divisions occur (next to the xylem) and that containing the young fibers (separated by tannin cells from the cambial region). The fusiform cambial cells of the fascicular area strikingly contrast with the short ray initials (plate 16, *B*).

No detailed studies were undertaken on the relative time of appearance of the new xylem and phloem cells. However, evidence obtained thus far indicates that both are formed approximately at the same time. The two tissues have an equally long growth period, but a xylem increment of a given season is much wider than the corresponding phloem increment. These observations agree with the conclusions drawn by Knudson (1916) from his studies on radial growth in *Vitis labrusca*.

Pattern of Cambial Reactivation in the Axis. To ascertain the spatial pattern of cambial reactivation in the axis, several cane pieces were studied in detail at bud break and the relative appearance of active cambium was also determined in the trunk and roots. Figure 8 shows the canes (really pieces of canes, called "canes" for convenience) that were used in this study. They were collected on the following dates of 1945: April 12 (fig. 8, *A*), April 17 (fig. 8, *B*), April 23 (fig. 8, *C*), and April 26 (fig. 8, *D-G*). The cane in figure 8, *A*, consisted of two parts: the lower, the main part of the cane (from the joint above *k* and downward), and the upper, a lateral branch on the main part of the cane. Both parts were one year old. The direct continuation of the main part of the cane was cut off between the levels *j* and *k* (in figure 8, *A*) long before the sample was taken, and the lateral part was trimmed at the cut end (above in the figure) at pruning time. The cane in figure 8, *C*, also has a joint (above *n*) but the one in figure 8, *B*, is all of one piece. The degree of development of the buds on the canes in figure 8, *A* and *B*, is indicated in the drawings. The bud below the level *d* in figure 8, *A*, and those below the levels *f* and *w* in figure 8, *B*, showed no signs of growth; all the others had leaves in various stages of unfolding. In the cane in figure 8, *C*, the longest lateral shoots were removed before the sketch was made. These were as follows: above level *a*, two shoots, 1.0 and 1.5 inches long, one with flower primordia; above level *g*, two shoots, 0.5 and 2.0 inches long, both with flower primordia; above level *n*, at the junction of two cane pieces, one shoot 2.0 inches long with flower primordia.

Sections for microscopic study were made from the three canes at the levels

indicated by the small letters in figure 8, *A* to *C*. To retain the proper relative orientation of the sections, a shallow longitudinal slit was made along one side of the cane and the exposed cells were dyed with safranine. Thus each section had a red spot on one side.

In the drawings, broken lines indicate the position of the sections that

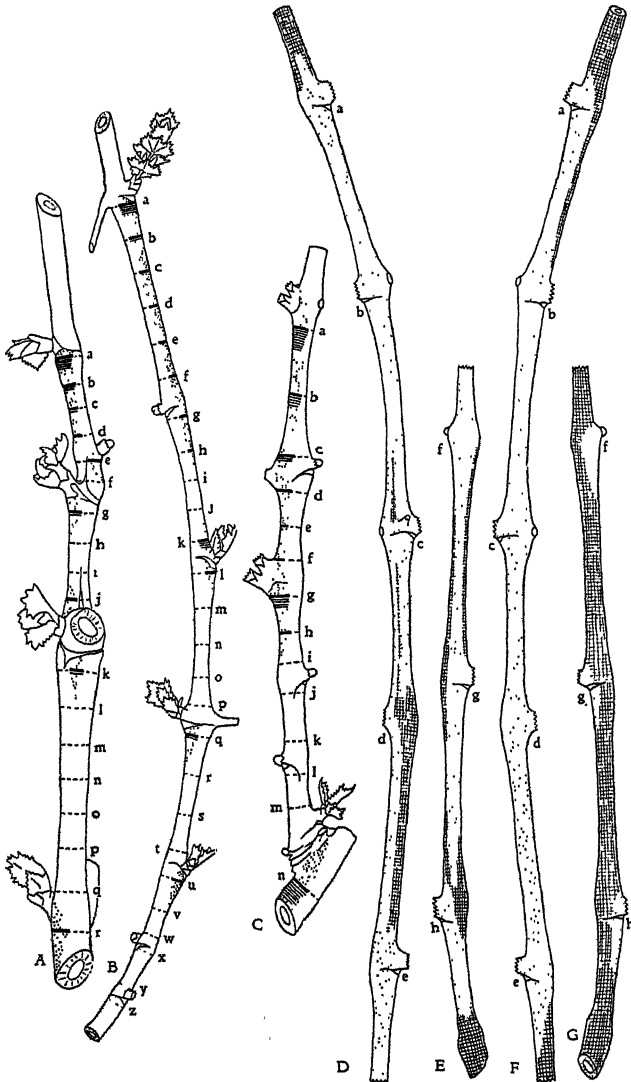


Fig. 8. Pieces of canes used in part of the study of cambial reactivation. The small letters in *A-C* indicate the levels at which the canes were sectioned. The cambium was active in the stippled parts in *A-C*; the solid lines denote the maximum number of divisions that had occurred at the different levels. *D* and *E*, Two pieces of the same cane separated from each other between the nodes *e* and *f*. *F* and *G*, The same two pieces of cane as in *D* and *E*, respectively, but turned 180° about their axes. The stippled areas in *D-G* show where the bark could be made to slip. In the cross-hatched areas the bark was firmly attached. The lateral shoots are not shown in *C-G* but are described in the text. (*A* × $\frac{2}{5}$; *B-G* × $\frac{1}{5}$.)

showed no cambial activity. Where a part of a line has been made solid, cambial activity was present but did not reach around the stem. One solid line at a given level signifies that only one division, at the most, had occurred here. If more than one cell was formed by the cambium, additional lines were drawn beneath the one located at the level of sampling. The total number of these solid lines at a given level corresponds to the maximum number of cells that were produced there by the cambium. The stippling delimits the area where the cambium was active.

Some of the sections made from the cane in figure 8, *A*, are outlined in cross-sectional views in figure 9. The small letters in the center of each section in figure 9 give the levels in the cane in figure 8, *A*, from which these sections were taken. In figure 9 only the xylem and the phloem were outlined. Leaf traces were clearly evident where, in the diagrams, the rays are represented by double lines. The broken line encircling the stem near the periphery gives the position of the inactive cambium; the solid lines that are continuous with the broken lines mark the active parts of this meristem. To emphasize the intermittent distribution of active cambium, the sectors containing such cambium were stippled.

Figure 8, *A* to *C*, shows that the initiation of the cambial activity is spatially related to buds. Usually this activity begins beneath the buds and then progresses downward. Sometimes the cambium becomes reactivated above a bud also, but only for a short distance (levels *j* in figure 8, *A*, and *k* in figure 8, *B*). Cambial activity is commonly absent beneath buds with no signs of growth (fig. 8, *B*, levels *g* and *x*; 8, *C* levels *j* and *l*). Since, as a rule, the uppermost bud on a cane is the first to begin growth, cambial activity also starts at the apex of the cane. Evidence of such apical dominance was present in the canes in figure 8, *A* to *C*: cambial activity that was induced beneath the uppermost buds had progressed to a greater distance downward than the activity associated with the lower buds.

Because cambial activity starts beneath a bud, the largest number of cells produced by the cambium, in the early stages of growth, occurs beneath a bud and this number decreases in the downward direction. The cross-sectional views in figure 9, *A* to *D*, show also that the lateral spread of cambial reactivation is greatest close beneath the bud and diminishes downward in the internode. In any given area with active cambium, the highest number of new cells occurs in the median portion of the area. Thus at level *a* in figure 8, *A*, six cells were formed in the middle of the arc of active cambium (see also figure 9, *A*), whereas toward the margins the number diminished to one. Figure 9, *E*, *G*, *J*, and *K* shows that although at the levels represented (fig. 8, *A*, levels *e*, *g*, *k*, and *r*) cambial reactivation had spread laterally to a considerable distance, it was absent at the nearest levels below (fig. 8, *A*, levels *f*, *h*, *l*, and 9, *F* and *H*). Thus near the levels *e*, *g*, *k* and *r*, in figure 8, *A*, the rather wide patches of active cambium were sharply isolated from each other. Figure 9, *I*, shows that at level *j* (see also fig. 8, *A*) where the cambium was reactivated in an upward direction from a bud, the lateral spread was very restricted.

Thus in the early stages of growth, active cambium occurs in discontinuous patches spatially related to the position of the buds on the canes. Gradually

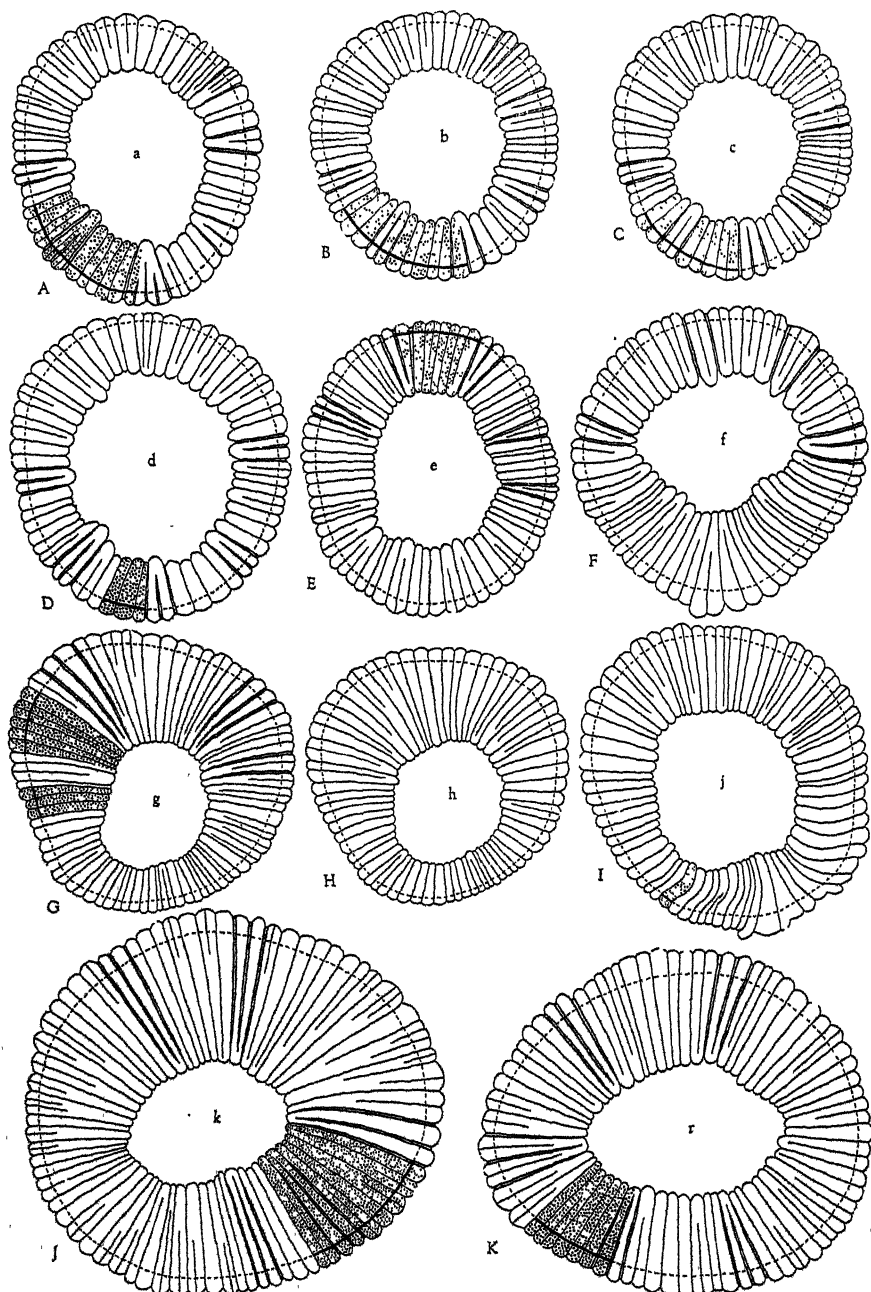


Fig. 9. Transverse sections of the cane illustrated in figure 8, A. The small letters a-h, j, k, and r, denote the levels in figure 8, A, from which these sections were taken. In all drawings the dormant cambium is indicated by a broken line, the active cambium by a solid line continuous with the broken one. The cane sectors containing active cambium are stippled. (All $\times 5$.)

reactivation spreads downward and laterally until the previously isolated patches merge. Figure 8, *D* to *G*, shows the distribution of active cambium during an advanced state of reactivation. The pieces depicted in this figure are all from one cane. Figure 8, *D* and *E*, shows this cane from one side, figure 8, *F* and *G*, from another. The piece in figure 8, *E* and *G*, was attached to the longer piece in figure 8, *D* and *F*, between the nodes *e* and *f*. The lengths and the developmental features of the lateral shoots that occurred at the various nodes on the cane in figure 8, *D* to *G*, were as follows: *a*, 6.0 inches, with flower buds; *b*, 10.5 inches, with flower primordia; *c*, 4.0 inches; *d*, 0.5 inches; *e*, 5.5 inches, with flower primordia; *f*, no growth; *g*, 0.5 inches; *h*, 3.0 inches.

The cane in figure 8, *D* to *G*, was not sectioned; instead, the position of the active cambium was located by determining the parts of the bark that could be made to slip without the aid of any instruments. These areas were marked by stippling in the drawings. In the cross-hatched portions the bark was firmly attached to the wood.

Most of the upper part of the cane (fig. 8, *D* and *F*) was in an active state. The lower part showed much active cambium on one side (fig. 8, *E*) but only small patches of it at the nodes on the other side (fig. 8, *G*). In the uppermost end of the cane, that is, in the portion located between the pruning cut and node *a*, cambial reactivation had spread upward from *a* for a short distance. The rest of this end was inactive.

When samples of phloem were taken from the main trunk, the presence or absence of cambial activity in the sampled areas was recorded. On April 17, when the cane shown in figure 8, *B*, was collected, a sample from the main trunk, taken 6 inches below the insertion of the main arms, showed no cambial activity. On April 25, the condition of the cambium in four samples taken on the trunk at various distances from the attachment of arms and downward was as follows: 8 inches, several cambial divisions; 17 inches, about two cambial divisions; 26 inches, one cambial division; 35 inches (5 inches from the ground), no cambial activity.

The dormant cambium of the roots has thinner walls than similar cambium of the aerial parts of the plant. Therefore the root bark can be forced off the root with relative ease during dormancy. The occurrence of cambial activity in the roots was determined by the use of sections only. In roots several years old, collected on May 2, no divisions had occurred in the cambium. On May 9 similar roots had cambial activity but not around the entire circumference.

Thus, according to the data just given, the grapevine shows a basipetal course of cambial reactivation characteristic of woody dicotyledons. The course of cessation of this activity was not considered in the present study.

Reactivation of the Phloem as Compared with that of the Cambium. The canes that were used for the study of cambial reactivation in 1945 were examined also with regard to the resumption of phloem activity. It became obvious at once that phloem reactivation started before any cambial divisions occurred in the same part of the plant. In all three canes shown in figure 8, *A* to *C*, connecting strands were present at all levels (although the callus was still massive in the peripheral parts of the phloem), whereas the cambial activity was very much localized in certain areas. The connecting strands in the canes in figure 8, *B* and *C*, had a uniform degree of development in all

sections, but in the cane in figure 8, *A*, sieve plates with connecting strands were more numerous in the upper sections than in the lower; and they were somewhat better developed on the sides of the cane where buds were present than on the opposite sides. This variation in the degree of phloem reactivation in the cane in figure 8, *A*, indicated some relation to the position of active buds. The cambial reactivation in the same cane, however, showed an incomparably more definite relation to the inception of growth in the buds.

Comparative studies on reactivation of cambium and phloem were repeated in 1946. Long pieces of canes were sampled at different levels and the condition of the phloem and cambium was recorded at these levels. The canes were taken from five-year-old vines that grew in a small experimental plot where the pruning operation was delayed until late in March. Thus, during dormancy and during the early part of the growth season, long canes were available and the samples could be taken at widely separated levels on the same canes.

The progress of reactivation was studied in two kinds of branches: in canes that were left on the plant until they were sampled for microscopic study, and in canes that were cut off, brought into a heated laboratory room, placed with the cut ends in water, and covered with a bell jar. (The induction of phloem reactivation in dormant canes by exposing them to room temperature was first studied by Wilhelm, 1880.)

Table 1 sums up the results obtained with the canes kept in the laboratory. Thirteen canes (canes 1 to 13 in table 1) were collected on January 4 (series 1 to 3) and one (cane 14 in table 1) on January 21 (series 4). One of the canes of the January 4 collection was sampled as soon as it was brought into the room, and the other 12 were kept indoors and were sampled on the dates given in the first column of table 1. Till January 16, inclusive (series 1, *A* in table 1), the uppermost nodes of successive canes were sampled. Since the buds in the group of 12 canes showed no signs of growth on January 16, these canes were sampled once more, beginning with the one that was sampled on January 5 (cane 2), and again the samples were taken at the uppermost nodes. The last of this set of samplings was made on January 23 when the buds had begun to grow (table 1, series 1, *B*).

Two of the canes of the January 4 collection, that were not sampled for the second time in series 1, *B* (canes 7 and 8) were used for comparing the state of reactivation of tissues at the different levels (table 1, series 2 and 3). Several nodes and median portions of the internodes below these nodes were sampled. The cane that was brought into the room on January 21 was treated in a similar manner (table 1, series 4).

As stated previously, the first column of table 1 gives the dates of samplings. This column also designates whether the sample consisted of a nodal or internodal piece of the cane. The numbers after the words "node" and "internode" in series 2 to 4 indicate the relative level of these samples on the cane, with the count beginning at the uppermost node. The second column specifies the number of days that the canes were in the room before the given sample was taken from them. (In series 1, certain groups of samples that gave the same results were combined in the table.) The condition of the buds immediately above the sampled node pieces is described in the third column.

TABLE 1
REACTIVATION OF PHLOEM AND CAMBIUM IN CANES KEPT IN A WARM ROOM,
SEASON OF 1946

Cane number, part of axis sampled, and date of sampling	Number of days in room	Condition of nearest bud above	Condition of cambium*	Maximal width of new growth, in microns	Condition of phloem†
<i>Canes collected Jan. 4:</i>					
Series 1, A, sampling of canes 1-13:					
Cane 1, internode sampled Jan. 4	0	Scales closed	—	0.0	—
Cane 2, node sampled Jan. 5	1	Scales closed	—	0.0	+
Cane 3, node sampled Jan. 6	2	Scales closed	—	0.0	—
Canes 4-7, nodes sampled Jan. 7-10	3-6	Scales closed	—	0.0	+
Canes 8-11, nodes sampled Jan. 11-14	7-10	Scales closed	—	0.0	++
Cane 12, node sampled Jan. 15	11	Scales closed	++	37.5	++
Cane 13, node sampled Jan. 16	12	Scales closed	—	0.0	++
Series 1, B, resampling of canes 2-6:					
Canes 2-3, nodes sampled Jan. 17-18	13-14	Scales closed	—	0.0	++
Cane 4, node sampled Jan. 19	15	Scales closed	++	50.0	++
Cane 5, node sampled Jan. 21	17	Just opening	—	0.0	++
Cane 6, node sampled Jan. 23	19	Just opening	+++	137.5	+++
Series 2, resampling of cane 7, Jan. 25:					
Node 1	21	2 mm out	++	112.5	+++
Internode 1	21	2 mm out	+	62.5	+++
Node 2	21	Just opening	++	137.5	+++
Internode 2	21	Just opening	++	87.5	+++
Node 3	21	5 mm out	+++	375.0	+++
Internode 3	21	5 mm out	+++	387.5	+++
Series 3, resampling of cane 8, Jan. 26:					
Node 1	22	6 mm out	+	37.5	+++
Internode 1	22	6 mm out	+	0.0	+++
Node 2	22	5 mm out	++	75.0	+++
Internode 2	22	5 mm out	++	12.5	+++
Node 3	22	5 mm out	+++	112.5	+++
Internode 3	22	5 mm out	+++	87.5	+++
<i>Cane collected Jan. 31:</i>					
Series 4, sampling of cane 14, Feb. 8:					
Node 1	18	5 mm out	++	12.5	+++
Internode 1	18	5 mm out	+	0.0	+++
Node 2	18	Scales closed	+	0.0	+++
Internode 2	18	Scales closed	—	0.0	+++
Node 3	18	Scales closed	+	0.0	+++
Internode 3	18	Scales closed	+	0.0	+++
Node 4	18	Scales closed	++	37.5	+++
Internode 4	18	Scales closed	++	62.5	+++
Node 5	18	3 mm out	+++	137.5	+++
Internode 5	18	3 mm out	+++	150.0	+++

* —, Cambium inactive.

+, ++, +++, Cambial cells enlarged or divided over about $\frac{1}{2}$ of, over about $\frac{2}{3}$ of, or over the entire circumference of the cane, respectively.

† —, No visible connecting strands and callus of maximal thickness.

+, Connecting strands in some callus masses and callus of maximal thickness.

++, Connecting strands in $\frac{1}{2}$ to $\frac{2}{3}$ of phloem thickness, callus slightly thinner than before.

+++ , Conspicuously thickened connecting strands present throughout most of the phloem and callus much thinner than during dormancy.

The method of estimating the degree of reactivation of the cambium and phloem (fourth and sixth columns in table 1) is explained in the footnotes to the table. The appearance of the phloem during dormancy and during the progress of reactivation has been described in detail in a foregoing part of this paper. In the material used for table 1, the phloem was not yet completely reactivated in the latest samples, but the process had advanced sufficiently to give a complete picture of its course in the different parts of the canes. The thickness of the new tissue produced by the cambium was measured where it was greatest (fifth column in table 1).

As table 1 shows, the first active cambium was observed in the sample taken 11 days after the canes were exposed to the specified laboratory conditions; but the reactivation was not uniform in the successive samples. Series 2 to 4 indicate that the apical dominance in the initiation of bud growth (that is, start of bud growth at the apex of the cane, then at successively lower levels) and the normal pattern of cambial reactivation (that is, its initiation beneath the uppermost buds, then at successively lower levels) was upset by exposure to laboratory conditions. The buds grew out at the bases of the canes soon after or simultaneously with those at the apices, and the cambium was more active at the bases than at the apices of the canes. In the two short pieces of series 2 and 3, cambial activity increased basipetally from node to node, with no direct relation to the degree of development of the associated buds, and in the lowermost part of the cane in series 2, radial growth was somewhat more advanced away from the bud (internode 3) than close to it (node 3). In the long cane in series 4, a region with no increase in radial thickness intervened between the nodes 1 and 4 (see the fifth column in table 1) and radial growth was much more advanced at the base of the cane than it was near the apex.

The change in the phloem marking the cessation of dormant state was evident in the cane sampled on January 5, the day after the canes were brought into the laboratory. In this sample, however, the connecting strands were very faint; they occurred in few sieve areas and these areas were scattered at different depths in the phloem; and the following sample showed no connecting strands. Beginning with cane 4 (sampled January 7), phloem reactivation developed consistently and reached its maximum, for this series of collections, after 19 days in the laboratory (cane 6, series 1, *B*).

In all the series of table 1, the distribution of the first visible connecting strands in the node showed no relation to the position of the bud; the nodes and the internodes below them showed no difference in the degree of reactivation; and the condition of the phloem was alike at the different levels of the same canes. Thus the pattern of phloem reactivation in this experiment did not coincide with that of cambial reactivation.

Table 2 gives the results obtained with canes collected at different times from vines growing out of doors. The method of sampling and recording of the data were as for series 2 to 4 in table 1, except that in cane 1 of table 2 no internodes were used. Since canes 2 and 3 were sampled on the same day and were similar with regard to the reactivation of buds, cambium, and phloem, the data pertaining to these canes were combined.

The first signs of cambial reactivation were observed in the cane sampled on March 23 (cane 6). In this cane and in the following one (cane 7), apical

dominance in bud development and the dependence of cambial reactivation on bud growth were clearly expressed. The first evidence of phloem reactivation preceded that of cambial reactivation. Between March 9 and March 30 the degree of phloem reactivation remained approximately the same; then it was speeded up. Canes 2, 3, 5, 6, and 7 showed a minor decrease in the intensity of reactivation toward their bases, but this decrease was so slight that it was not recorded in the table. In cane 9, on the contrary, the connecting strands were distinctly more conspicuous at the base than at the top, but here the upper part of the cane showed necrosis in the bark on one side. This abnormality did not seem to affect the reactivation of the cambium, however.

Thus the present studies revealed no close relation between phloem reactivation and bud growth. A basipetal progress of activation was indicated but the evidence of this course quickly disappeared. The reactivation of the phloem definitely preceded that of the cambium. This was true of the canes and also of the older stem parts, whereas the roots showed no complete dormancy in the phloem.

Seasonal Cycle of Phloem Activity.¹¹ In the present study the most detailed investigation of seasonal changes in phloem were made on canes of the Sultanina variety. The diagram in figure 10 gives an approximate picture of the yearly cycle in the transformations in the phloem of such canes. Since in its initial stages the activity of the cambium shows a basipetal progression in the axis, different levels of a given cane show the beginning of cambial divisions at different times. The samples used for figure 10 were taken from various parts of the canes but mostly from the median and near basal ones. Naturally, the weather conditions and other environmental factors also affect the dates when the various phenomena occur in the tissues of a plant. Figure 10 is based on data compiled during two years, 1945 and 1946. In 1945, growth began somewhat later than in 1946; and dormancy was reached much later in 1945 than in 1946.

The diagram in figure 10 may be used to follow the changes in a given increment of the phloem through its two years of existence; and it also may serve to illustrate the changes in activity in the phloem as a whole during one season. Cambial activity, lasting about two and a half months, gives rise to a phloem increment. This activity is localized at the start but becomes rather general in a cane toward the end of March, that is, when the leaves of the axillary buds on the canes are unfolding. The first sieve tubes are discernible in the latter part of April. Cambial divisions cease and most of the sieve tubes are mature by the middle of June. The narrow-celled phloem, which is produced at the end of the growth period and constitutes only a small part of the phloem increment as a whole, remains thin-walled and, apparently, immature until late in August. (Figure 10 disregards this late maturation of the last part of a phloem increment.)

The newly formed phloem remains fully active and shows no morphologic changes until after the middle of October. At this time callus—the dormancy callus—begins to develop, usually in the peripheral portions of the phloem

¹¹ In this discussion the identification of the phloem in the various states (namely, active, inactive, partly active, and fully active) is based solely on such morphologic characteristics as were described in detail in earlier parts of this paper.

TABLE 2
REACTIVATION OF PHLOEM AND CAMBIUM IN CANES ON VINES GROWING OUT OF
DOORS, SEASON OF 1946

Cane no., date of sampling, and part of axis	Condition of nearest bud above	Condition of cambium*	Maximum width of new growth, in microns	Condition of phloem†
Cane 1, sampled March 2:				
Node 1.....	Scales closed	—	0.0	—
Node 4.....	Scales closed	—	0.0	—
Node 8.....	Scales closed	—	0.0	—
Canes 2 and 3, sampled March 9:				
Nodes and internodes 1‡ and 1‡.....	Just opening	—	0.0	+
Nodes and internodes 4 and 5.....	Scales closed	—	0.0	+
Nodes and internodes 7 and 9.....	Just opening	—	0.0	+
Nodes and internodes 10 and 13.....	Scales closed	—	0.0	+
Cane 4, sampled March 16:				
Node and internode 8.....	125 mm out	—	0.0	+
Node and internode 15.....	Scales closed	—	0.0	+
Cane 5, sampled March 16:				
Node and internode 7.....	Shoot with active buds	—	0.0	+
Node and internode 8.....	Just opening	—	0.0	+
Node and internode 10.....	Just opening	—	0.0	+
Node and internode 12.....	Scales closed	—	0.0	+
Node and internode 14.....	Scales closed	—	0.0	+
Cane 6, sampled March 23:				
Node 1.....	60 mm out	++	25.0	+
Internode 1.....	60 mm out	+	0.0	+
Node 6.....	Just opening	—	0.0	+
Internode 6.....	Just opening	—	0.0	+
Node 11.....	Scales closed	—	0.0	+
Internode 11.....	Scales closed	—	0.0	+
Cane 7, sampled March 23:				
Node 1.....	140 mm out	++	37.5	+
Internode 1.....	140 mm out	—	0.0	+
Node 5.....	140 mm out	++	0.0	+
Internode 5.....	140 mm out	—	0.0	+
Node 6.....	Just opening	—	0.0	+
Internode 6.....	Just opening	—	0.0	+
Node 14.....	Scales closed	—	0.0	+
Internode 14.....	Scales closed	—	0.0	+
Cane 8,§ sampled March 30:				
Node 1.....	1st leaf 2 cm long	+	12.5	++
Internode 1.....	1st leaf 2 cm long	—	0.0	++
Node 4.....	Scales closed	—	0.0	++
Internode 4.....	Scales closed	—	0.0	++
Cane 9, sampled April 10:				
Node 1.....	2 cm out	++	137.5	++
Internode 1.....	2 cm out	++	12.5	++
Node 2.....	No bud	—	0.0	++
Internode 2.....	No bud	—	0.0	++
Node 8.....	1.5 cm out	++	125.0	++
Internode 8.....	1.5 cm out	—	0.0	++
Node 16.....	Just opening	+	0.0	++
Internode 16.....	Just opening	—	0.0	++

* —, Cambium inactive.

+, ++, +++, Cambial cells enlarged or divided over about 1/4 of, over about 2/4 of, or over the entire circumference of the stem, respectively.

† —, No visible connecting strands and callus of maximal thickness.

+, Connecting strands in the innermost part of the phloem and callus of maximal thickness.

++, Connecting strands in 1/4 to 3/4 of phloem thickness, callus slightly thinner than before.

+++, Conspicuously thickened connecting strands present throughout most of the phloem, and callus much thinner than during dormancy.

‡ The first set of figures after the words "node" and "internode" refers to nodes and internodes on cane 2, the second set to those on cane 3.

§ Between March 23 and March 30 the canes were cut back in a pruning operation and therefore the upper ends of the canes were not available on March 30 and later.

first, then closer to the cambium as well. This initial development of the dormancy callus is very uneven in different parts of the same section and in different sections sampled successively. After the first frost and the concomitant leaf fall (commonly late in November, but sometimes early in December), the dormancy callus suddenly appears rather generally throughout the phloem. Thus this tissue becomes dormant; and it remains so for approximately three and a half months.

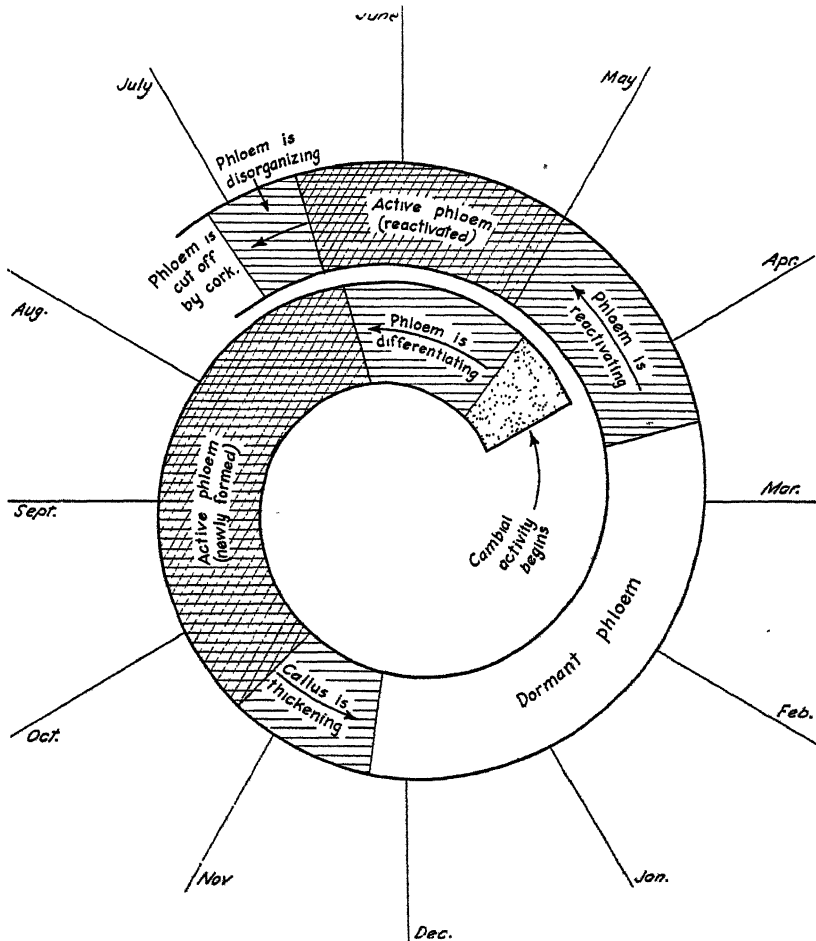


Fig. 10. Diagram illustrating the seasonal changes in one increment of secondary phloem in a cane. (This diagram was designed by Dr. L. K. Mann.)

In the middle of March, connecting strands become visible again and the callus thins down, first near the cambium, then also farther outward. By the end of April or early in May, all the sieve tubes acquire the appearance of active elements, such as they had before the inception of dormancy in the preceding season. Some few sieve tubes near the periphery may fail to return to the active state. Such elements become completely functionless and are

either crushed or filled with tyloses. The reactivated phloem retains the same morphologic features until the second half of June. At this time the first indications of final disorganization—with or without formation of definitive callus—become perceptible. Like the change preceding dormancy in the fall, this final disorganization (preparatory to the formation of dead bark) does not occur uniformly and may be delayed until very shortly before the cork is formed. The latter arises early in July and causes the death of most of the phloem increment that was formed the year before. Since the cork cambium arises within this increment, some of the old phloem is left connected to the newly formed phloem. The sieve tubes of such a remnant of the old phloem become obliterated.

During one season, the course of transformations in the phloem as a whole has the following notable features. The reactivation of the phloem increment that originated in the preceding season begins while the cambium is still inactive. At this time the upper axillary buds are breaking through the scales, but the leaves are not yet unfolding. Cambial activity starts about two weeks later; but before much of the new phloem has been formed, the old phloem is fully reactivated. The latter tissue constitutes—in partly and in fully active states—the principal portion of the food-conducting tissue while the axillary shoots elongate and their leaves unfold and while the flowers develop from their primordia. (The latter are laid down during the preceding summer; according to Winkler and Shemsettin, 1937.) Of course, the amount of new phloem is constantly increasing in the meantime and early in June, when the berries begin to grow (Winkler and Williams, 1936) and the next-season cluster primordia are initiated (Winkler and Shemsettin, 1937), more than half of the new phloem increment is mature. When the berries are of about one half to two thirds of their mature size, the old phloem begins to show signs of disorganization. In the meantime the formation of the new phloem is completed, and after the cork is formed this phloem remains, during the rest of the season, as the only food-conducting tissue. Its morphology does not change until late in October when occasional signs of approaching dormancy become apparent. Finally, at leaf fall, full dormancy is attained. Thus, fully active phloem is available in the canes from early May till past the middle of October; partially and fully active phloem together, from the middle of March till late in November.

In the discussion just given, the phloem is presented as becoming functionless after existing for two seasons. Though a given phloem increment usually functions during two seasons, it may function during three and even four seasons. Such longevity is uncommon in canes but is often encountered in trunks; it is more pronounced in some varieties than in others. Judging from the present observations, a phloem increment that is active during several seasons becomes dormant every winter and is reactivated every spring.

In order that the canes might be compared with other parts of the vines, some samples were collected in 1945 from trunks, roots, and new shoots. The bases of the latter had fully formed primary phloem, with lignified proto-phloem fibers, early in May. By the middle of June cambial activity had ceased and most of the secondary phloem was mature. In the shoots, as in the cane, the maturation of the narrow-celled phloem that completed the yearly incre-

ment of secondary phloem was delayed until late in August. When cork appeared in the canes it was also evident in the shoots, although only at their bases. From here this tissue developed acropetally and cut away all the proto-phloem and sometimes deeper lying phloem parts as well. Complete dormancy was observed in shoot samples collected early in December.

Because of the basipetal course of cambial reactivation in the vine as a whole, the trunks showed a delay (as compared with the canes) in the commencement of cambial divisions. In 1945 the canes exhibited active cambium during the first week of April. The first bark sample from a trunk was taken at the top of the trunk on April 17; it showed no cambial activity. On April 25 cambial divisions reached the middle of the trunk. On May 2 one vine showed active cambium 4 inches above the ground level; another did not. On May 9 cambial activity was observed in the roots. Thus, judging from the few samples examined, some three to four weeks had elapsed between the inception of radial growth in the canes and that in the bases of the trunks.

The samples of April 17 and 25, taken at different levels of trunks and exhibiting different degrees of cambial reactivation, were uniform with regard to the state of phloem reactivation. They were similar also to the cane samples taken at the same time in having partly reactivated phloem, with connecting strands and somewhat thinner callus than during dormancy. Evidently, phloem reactivation in both canes and trunks occurred more uniformly than cambial reactivation.

The disorganization of the phloem that preceded cork formation began irregularly in both canes and trunks, that is, the successive samples showed no uniform progression in the development of this phenomenon. Similarly, the initiation of cork lacked uniformity in the time of appearance and in the spread around the axis. In general, the cane and trunk samples showed new cork at similar times. The samples showed complete dormancy on December 3, but the first evidence of callus thickening was found on October 29 in the canes and on November 19 in the trunks.

As was pointed out previously, the samples of roots showed cambial reactivation some four weeks later than those of canes. Samples collected during the winter had no dormancy callus, but some exhibited thicker callus (with obvious connecting strands, however) at this time than during the summer. The cork was formed more irregularly in the root samples than in the others. Some three- to four-year-old roots still had some pericycle. The presence of the latter and the accumulation of nonfunctioning phloem, previously described in this paper, suggests that cork formation may be omitted in some seasons, at least in parts of roots.

Variations in the Thickness of Phloem. To complete the picture of phloem growth in *Vitis vinifera*, some data were gathered on the thickness and composition of bark in the varieties Sultanina and Ohanez. These data serve as a basis for tables 3 to 6 and offer comparisons of the following items: the bark of young and old growth on similar vines (table 3); bark of trunks of different varieties (tables 3 to 5); and bark of trunks of different ages (table 6). The tables consider the thickness of bark, the number of annual increments, and the numbers of sieve-tube and fiber bands.

In the canes the bark was measured in four places around the circumference.

The bark samples from the trunks were $\frac{1}{2}$ to 1 cm in tangential extent and were measured either in two (data for tables 3 and 4) or four (data for table 6) places. In 1945 (tables 3 to 5) the samples were collected successively through part of the season, beginning in early spring and ending just before the formation of new cork. In 1946 (table 6) all collections were made in February while the vines were still dormant.

Since the samples of 1945 were collected before that season's growth was completed, the 1945 phloem was excluded from the study. The "number of annual increments" in tables 3, 4, and 6 included the outermost (the oldest) increment, which usually was incomplete because a part of it was cut off by cork formation during the preceding season. If the oldest increment was represented by one band of fibers and a narrow band of late phloem, or by the late phloem alone, such partial increment was not counted. If, however, the late phloem was associated with at least a part of a band having wide sieve tubes, the combination was included in the count. The presence of a fragment of an older increment on the outer periphery of the phloem indicated that the younger increments were present in their entirety. Such complete annual increments are analyzed in table 5. Sometimes the remnants of the oldest increments were obscured by changes associated with the loss of function and the initiation of cork cambium. In such instances, the outer limits of the outermost complete increment remained uncertain.

Table 3 gives data on bark thickness in the cane and trunk of Sultanina and in the trunk of Ohanez. The minimal and maximal values in the sixth, seventh, ninth, and tenth columns are not averages of observations on one sample, but represent certain individual observations. For example, among 30 measurements on the trunks of Sultanina vines, 1 gave the minimal value of 0.55 mm for total bark thickness. The averages in the eighth and eleventh columns are based on all observations on a given set of samples.

Judging from table 3, the one-year-old canes of Sultanina have thinner bark than the trunks. In the more or less elliptical transection of a cane, the vascular tissues are uneven in thickness: they are usually thinner at the extremities of the shorter diameter, thicker at those of the longer diameter. The uneven thickness of the xylem and phloem are visible in plate 5, *B*, though the outline of this cane deviates only little from the circular. In less rounded canes the variations in tissue thickness may be even greater. This anatomic feature explains the difference between the minimal and maximal bark thicknesses of canes in table 3. The trunk samples were more uniform in thickness, with the exception of 1 among 15, which was taken in a deep groove upon a trunk. The bark was very thin at the bottom of the groove. The Ohanez trunk samples in table 3 had thicker bark than those of Sultanina, though the trunks of the latter were older. Ohanez forms in general a thicker trunk than Sultanina.

Table 4 gives information on the relative thickness of bark in different parts of the same trunk. The samples from Sultanina, vine 1, indicate considerable uniformity in bark thickness at different levels of the trunk. The samples taken around the circumference of the trunk of vine 2 of Sultanina are all, except one, rather similar in thickness. The exception was the thin bark at the bottom of a deep groove previously mentioned. The Ohanez vines also showed little variation in bark thickness in the different parts of the trunk.

Sultanina, vine 2, and Ohanez, vine 2, showed the same average number of annual increments of phloem, though the former had a thinner bark than the latter. Judging from this observation, the total bark thickness depends not only upon the number of annual increments, but also upon the width of each increment. Indeed, a thin bark sample may have more annual increments than a thick one. (Compare, for example, samples 8 and 9 in Sultanina, vine 2, and the various samples in Ohanez, vine 1.) Many observations indicate that the accumulation of several annual increments and the repeated reactivation of the same increment is particularly common in parts of the stem where phloem growth is abnormally weak.

TABLE 3
COMPARISON OF BARK THICKNESS IN CERTAIN VARIETIES OF *Vitis vinifera*,
SEASON OF 1945

Variety	Characteristic of plant part that was sampled		Number of vines	Number of bark samples	Number of annual increments of phloem without the current growth			Thickness of bark* in mm		
	Kind	Age, in years			Minimum	Maximum	Average	Minimum	Maximum	Average
Sultanina.....	Internode of cane	1	10	11	1	1	1.0	0.29	0.73	0.50
Sultanina.....	Trunk	11	4	15	1	3	2.2	0.55	1.40	1.03
Ohanez.....	Trunk	8	2	17	2	4	2.9	1.30	2.55	1.84

* Includes cork, which is about 0.05 mm thick.

To obtain detailed information on the width of the individual annual increments and their composition, the trunk bark of the vines used in tables 3 and 4 and of some others was analyzed in table 5. The 18 bark samples of Sultanina used for table 5 were taken from 7 different vines as the table shows, vines 1 to 4 having eleven-year-old trunks, vines 5 to 7 four-year-old trunks. The 2 Ohanez vines used for sampling had eight-year-old trunks.

In table 5 only the complete annual increments were considered, that is, those increments whose outer limits could be determined by the presence of the late phloem of the next youngest increment. The values for thickness are given for all complete annual increments of each bark sample together and for each increment separately. The numbers of the sieve-tube and fiber bands also were determined for the sum of the complete increments of a given bark sample and for each increment separately. Annual growth always starts and ends with a sieve-tube band (plates 7, A; 8, A; and 16, A). Therefore, the number of fiber bands in a yearly increment is always one less than that of the sieve-tube bands. If the last sieve-tube band was very narrow and was composed of late phloem only (as in figure 7, B to D), it was not counted as an entire band. In table 5, the presence of such a narrow sieve-tube band was recorded by means of a plus sign following the number for the sieve-tube bands. The distribution of plus signs in table 5 indicates the frequent formation of a narrow band of late phloem after the last fiber band.

Table 5 shows the several features which cause the greater thickness of trunk bark from Ohanez, as compared with similar bark from Sultanina. Each

TABLE 4
COMPARISON OF BARK THICKNESS AT DIFFERENT LEVELS AND AT DIFFERENT POSITIONS
AROUND THE CIRCUMFERENCE OF TRUNK, SEASON OF 1945

Variety, vine no., and sample no.	Location of samples on trunk	Number of annual increments of phloem without the current growth	Thickness of bark,* in mm
<i>Sultanina:</i>			
Vine 1:			
Sample 1	32 inches from the ground	2.0	1.15
Sample 2	23 inches from the ground	2.0	1.10
Sample 3	14 inches from the ground	2.0	1.05
Sample 4	5 inches from the ground	2.0	1.05
Average		2.0	1.09
Vine 2:			
Sample 5	2 feet from the ground	2.0	0.93
Sample 6	60° to the right of sample 5.	3.0	1.10
Sample 7	120° to the right of sample 5	2.0	0.95
Sample 8	180° to the right of sample 5.	3.0	0.65
Sample 9	240° to the right of sample 5.	2.0	1.05
Sample 10	300° to the right of sample 5	3.0	0.95
Average		2.5	0.94
<i>Ohanez:</i>			
Vine 1:			
Sample 1	4 feet from the ground.	3.0	2.00
Sample 2	90° to the right of sample 1.	2.5	1.75
Sample 3	180° to the right of sample 1.	2.0	1.75
Sample 4	270° to the right of sample 1.	3.0	2.13
Sample 5	1 foot from the ground.	3.0	2.15
Sample 6	90° to the right of sample 5.	4.0	1.83
Sample 7	180° to the right of sample 5.	3.0	1.80
Sample 8	270° to the right of sample 5.	4.0	1.88
Average		3.1	1.91
Vine 2:			
Sample 9	4 feet from the ground.	2.0	1.35
Sample 10	90° to the right of sample 9.	3.5	1.88
Sample 11	180° to the right of sample 9.	2.5	1.30
Sample 12	270° to the right of sample 9.	2.0	1.70
Average		2.5	1.56

* Includes cork, which is about 0.05 mm thick.

year Ohanez produces a higher number of sieve-tube and fiber bands of a greater total thickness than Sultanina. Furthermore, Ohanez accumulates, on the average, a higher number of annual increments than the other variety.

In the discussion of tables 3 to 5 the condition of the phloem—that is, whether all or only part of it was in functioning state—was not mentioned. However, observations on barks of various thicknesses indicated, firstly, that a trunk with an exceptionally thick bark (table 4, Ohanez, vine 1; see also

TABLE 5

COMPARISON OF THE AMOUNT OF PHELOEM PRODUCED DURING ONE YEAR ON THE TRUNKS
OF CERTAIN VARIETIES OF *Vitis vinifera*, SEASON OF 1945

Variety, vine no., age of vine trunk, and sample no.	Complete annual increments		Total number of bands with the different cells		Number of sieve-tube bands in the different years			Thickness of phloem in each annual increment, in mm.		
	Num- ber	Thick- ness, in mm	Sieve tubes	Fibers	1942	1943	1944	1942	1943	1944
<i>Sultana</i> :										
Vine 1, 11-yr.-old trunk:										
Sample 1	3	1.12	7	6	3	2	3	0.47	0.36	0.29
Sample 2	1	0.70	4+*	4			4+*			0.70
Sample 3	1	0.73	5	4			5			0.73
Sample 4	1	0.70	4+	4			4+			0.70
Sample 5	1	0.62	4+	4			4+			0.62
Sample 6	1	0.88	5	4			5			0.88
Vine 2, 11-yr.-old trunk:										
Sample 7	2	0.81	6+	5		3	3+		0.39	0.42
Sample 8	3	1.06	7	5	3+	2	2	0.49	0.34	0.23
Sample 9	1	0.60	4	3			4			0.60
Sample 10	3	0.78	6+	4	3	1	2+	0.39	0.10	0.29
Sample 11	1	0.70	4+	4			4+			0.70
Sample 12	2	0.58	3+	2		1	2+		0.16	0.42
Vine 3, 11-yr.-old trunk:										
Sample 13	1	0.60	3+	3			3+			0.60
Sample 14	2	0.81	5	4		2+	3		0.42	0.39
Vine 4, 11-yr.-old trunk:										
Sample 15	2	0.78	5	4		2+	3		0.31	0.47
Vine 5, 4-yr.-old trunk:										
Sample 16	1	0.45	3	2			3			0.45
Vine 6, 4-yr.-old trunk:										
Sample 17	1	0.65	4	3			4			0.65
Vine 7, 4-yr.-old trunk:										
Sample 18	1	0.65	3+	3			3+			0.65
Average per increment	..	0.47	2.9	2.4		1.9	3.4	0.45	0.30	0.54
<i>Ohanez</i> :										
Vine 1, 8-yr.-old trunk:										
Sample 1	3	2.40	12+	12	4+	4+	4+	0.73	0.86	0.81
Sample 2	3	1.89	11+	11	3+	4+	4+	0.57	0.75	0.57
Sample 3	3	1.81	10	9	3+	3+	4	0.49	0.82	0.70
Sample 4	2	1.54	9+	8		3+	6+		0.60	0.94
Sample 5	3	2.13	12+	11	4+	4	4+	0.73	0.85	0.75
Sample 6	3	2.15	12+	11	4	4+	4+	0.65	0.75	0.75
Sample 7	3	1.46	9+	9	3+	3+	3+	0.47	0.47	0.52
Sample 8	2	1.25	9+	8		4+	5		0.55	0.70
Sample 9	3	1.61	9+	9	3+	3+	3+	0.57	0.55	0.49
Sample 10	2	1.56	9	8		4+	5		0.78	0.78
Sample 11	2	1.25	7+	7		3+	4+		0.60	0.65
Vine 2, 8-yr.-old trunk:										
Sample 12	2	1.30	8+	7		4	4+		0.55	0.75
Sample 13	2	1.04	7+	7		3	4+		0.47	0.57
Sample 14	3	1.63	9	8	3+	3+	3	0.49	0.65	0.49
Sample 15	2	1.17	6+	5		2+	4+		0.44	0.73
Sample 16	2	1.41	8+	8		4+	4+		0.68	0.73
Sample 17	2	1.41	8+	7		3+	5		0.60	0.81
Average per increment	..	0.64	3.7	3.5	3.4	3.4	4.1	0.59	0.62	0.69

*+, Only a thin layer of late phloem occurring on the inside of the latest fiber band.

plates 8 and 9) may have a larger amount of nonfunctioning phloem than a similar trunk with thinner bark (table 4, Ohanez, vine 2); and, secondly, that thicker, older bark may have a wider layer of functionless phloem than the thinner, younger bark. To check these observations in some detail, the data for table 6 were assembled. The variety Ohanez was chosen for this study because,

TABLE 6
COMPARISON OF THICKNESS AND COMPOSITION OF PHLOEM IN OHANEZ PLANTS OF DIFFERENT AGES, SEASON OF 1946

Number of annual increments	Number of fiber bands	Thickness of phloem, without cork, in mm						Per cent of functioning phloem					
		Total			Functioning			Functionless					
		Minimum	Maximum	Average*	Minimum	Maximum	Average*	Minimum	Maximum	Average*	Minimum	Maximum	Average*
Cane from 3-year-old trunk													
1.....	1-5	0.34	0.78	0.50	0.26	0.73	0.43	0.05	0.08	0.07	76.5	93.6	86.0
Trunk, 3 years old													
1.....	3-5	0.36	0.73	0.56	0.33	0.65	0.51	0.03	0.08	0.05	88.6	92.9	91.1
1.....	3-5	0.36	0.66	0.53	0.31	0.57	0.46	0.05	0.08	0.07	83.0	91.9	80.8
Average.....	0.55	0.49	0.06	89.1
Trunk, 5 years old													
1.....	6-7	0.88	1.20	1.06	0.72	1.15	0.91	0.05	0.23	0.15	77.9	95.8	85.8
1.....	5-6	1.01	1.12	1.07	0.88	1.02	0.95	0.10	0.21	0.13	80.7	91.1	88.8
1.....	6-7	1.09	1.25	1.14	0.96	1.09	1.03	0.05	0.18	0.12	84.2	95.4	90.4
1.....	6-7	1.23	1.25	1.24	0.96	1.07	1.02	0.13	0.23	0.23	76.8	85.6	82.3
2.....	5-8	1.51	1.69	1.59	1.38	1.51	1.43	0.13	0.13	0.16	88.8	91.4	89.9
Average.....	1.22	1.06	0.16	88.5
Trunk, 8 years old, from arbor vines planted 5 x 6 feet apart													
1.....	5-7	1.27	1.35	1.31	0.96	0.99	0.98	0.31	0.36	0.33	73.3	76.2	74.8
1.....	7-11	1.30	2.05	1.69	0.93	1.35	1.19	0.08	0.78	0.50	72.0	93.8	70.4
2.....	6-7	1.12	1.17	1.14	0.89	1.04	0.94	0.13	0.23	0.20	69.5	88.9	82.5
Average.....	1.38	1.04	0.34	75.4
Trunk, 8 years old, from arbor vines planted 20 x 24 feet apart													
2.....	6-8	1.20	1.48	1.32	0.97	1.22	1.08	0.21	0.26	0.23	80.8	83.2	81.8
1.....	7-7	1.40	1.45	1.45	1.32	1.36	1.36	0.08	0.10	0.10	93.2	94.3	93.8
1.....	6-7	1.38	1.48	1.42	1.20	1.30	1.24	0.10	0.26	0.18	82.4	92.9	87.3
Average.....	1.40	1.23	0.17	87.9

* Average of four measurements.

in addition to the plants with the eight-year-old trunks, a few vines with younger trunks were available for sampling. Each trunk-bark sample in table 6 was obtained from a different plant. The cane sample, which was taken from a plant with a five-year-old trunk, was included in table 6 to represent a one-year-old axis.

Table 6 shows that older axes have thicker phloem than the younger ones, with a higher number of sieve-tube and fiber bands. (Only the latter are recorded in this table; usually, the number of sieve-tube bands may be calculated by adding one, or a plus sign to the number of fiber bands.) Furthermore, as the trunk ages, it tends to accumulate more than one annual increment of phloem in the bark. The cane and the three-year-old trunks showed much less nonfunctioning phloem than the older trunks. The functioning phloem of the younger axes was, however, also considerably thinner than that of the older ones. In other words, the greater thickness of the functionless phloem in the older trunks was compensated for by the greater total thickness of the bark. As a result of these relations, the per cent of functioning phloem was comparable in most axes. Two trunks from the arbor vines in a close planting had a lower per cent of functioning phloem, but the number of samples was not high enough to permit the conclusion that the close spacing had anything to do with the increase in the relative amount of functionless phloem.

Seasonal Variations of Starch Content in Canes. This study was made in a qualitative way, by the use of iodine, on samples collected in 1945. Early in March, before the break of dormancy, the canes showed large amounts of starch in the parenchyma, the septate fibers, and the rays of xylem and phloem. The starch grains in the phloem cells were markedly smaller than those in the xylem. The cambial cells in the fascicular region contained no starch (nor tannin), but those within the ray resembled mature ray cells with regard to starch and tannin content. The first obvious signs of starch removal appeared when the cambium became active; they were limited to the immediate vicinity of the active cambium on the phloem side. In sections stained with iodine, the zone of active cambium now appeared as a light line dividing the xylem and phloem, both of which were still rich in starch. As the cambial activity spread around the circumference of the cane, starch progressively disappeared in the same direction. In the meantime, where cambial activity started first, the starch became depleted in successively more peripheral regions of the phloem. Early in May, at the time when the old phloem completed its reactivation (fig. 10), all bark appeared to be free of starch. A conspicuous depletion of starch now began in the xylem. In this tissue the starch content reached its lowest level (although not disappearing completely in the samples used) by the middle of June, at the time when the new phloem and xylem were fully formed.

After the middle of June the starch content of the xylem began to rise again, and by the end of this month it became evident in the new xylem. Soon after, the starch appeared in the phloem, but not in the parts that were partially disorganized and were to be cut off by the cork somewhat later (fig. 10). During the second week of July, starch accumulation almost reached the cambium on the xylem side. The youngest cells near the cambium developed large amounts of starch in early September, that is, shortly after the narrow layer of the

phloem, formed at the very last, became mature. (See "Seasonal Cycle of Phloem Activity," p. 257.)

The observations just reported agree rather well with the data on carbohydrate metabolism in *Vitis vinifera* obtained by Winkler and Williams (1945, fig. 1). These writers found a rapid drop in starch content when growth began, with the lowest level occurring in June or July, depending on the part of the plant tested. After this final drop, the starch content rose till it reached a peak early in October. Winkler and Williams (1945) observed a second drop in starch content during dormancy, with a difference of some 4 to 6 per cent (based on residual dry weight) between the fall maximum and the winter minimum. The method of testing for starch used in the present study was not delicate enough to detect the winter fluctuation in the starch content.

Regarding the relative time and degree of starch depletion in the bark and wood, Winkler and Williams (1945) also found an indication of primary utilization of bark reserves and observed relatively large amounts of carbohydrates in the wood even at the summer minimum.

DISCUSSION

The present study confirms the observations of early workers (Wilhelm, 1880; Lecomte, 1889; Strasburger, 1891; Hill, 1908) that the phloem of grapevine functions more than one season, becoming dormant during the winter and being reactivated in the spring. The present investigation proves in addition that the reactivation of phloem begins before the division of cells is resumed in the cambium.

Considering the plant as a whole, the occurrence of dormancy is not necessarily a constant character of *Vitis* phloem. In the present study complete dormancy was observed in the aerial parts of the vine, but the roots had non-dormant sieve tubes during the winter. Furthermore, the dormant state was attained in the stem parts only after the first frost. It is conceivable that in a milder climate than that of California, or in certain seasons, even the aerial plant parts could show incomplete dormancy or none at all.

The initial cambial activity in *Vitis* is closely related (as in other dicotyledons) to bud growth, depending on the reactivation of the buds (and the concomitant production of growth hormones); it begins beneath the earliest apical buds and progresses in a basipetal direction from the branch apices toward the main trunk and roots. No comparable localization and dependence on bud growth was observed, in the present study, with regard to the initiation of phloem activity. Some data suggested a basipetal progression of phloem reactivation, at least in the canes. More definite information regarding the progress of this phenomenon, especially in the plant as a whole, is not yet available.

The peculiar changes that occur in the sieve tubes as they become dormant and as they are reactivated, bear upon the present concept of the functioning sieve tube. According to this concept, the protoplast of a mature (functioning) sieve tube is in a denatured or premortal state. (See review by Esau, 1939.) In *Vitis* the "denatured protoplasts" of the sieve tubes show a considerable longevity and, during this long life, remain capable of performing vital functions, at least such as are involved in the repeated deposition and removal of

callose. The occurrence of prolonged activity in an enucleate protoplast of a highly evolved plant is a singular phenomenon, indeed, and a speculative assumption seems relevant that the normal nucleate protoplasts of the companion cells have some relation to the sieve-tube protoplasts (Esau, 1947).

The occurrence of phloem reactivation gives a unique opportunity for testing with reasonable exactness the anatomist's notion of a functioning sieve tube. According to the morphologic concept (Esau, 1939, p. 403), one which has emerged mainly from studies of plants showing no phloem reactivation, an active sieve-tube element is enucleate; it contains parietal cytoplasm and a vacuole having the dispersed slime—usually accumulated on the sieve plates in cut material—as one of its components; it has only small amounts of callose on the sieve plate and has very prominent connecting strands in the sieve-plate pores; it has starch grains, if their presence is characteristic for a given species; and it is associated with active companion cells. The appearance of the reactivated phloem of *Vitis* supports this concept: the reactivated sieve tubes have the features just described. These features are exhibited by the sieve tubes (the reactivated and the newly formed elements combined) throughout the period of active growth of the plant (fig. 10). The interpretation seems well justified that the sieve-tube elements which have such characteristics are functioning elements.

The presence of prominent connecting strands in the functioning sieve tubes deserves special emphasis. Though the large callus masses may offer no obstruction to the flow of materials through the sieve-plate walls, and though the connecting strands may play no direct role in the translocation of materials through the sieve tubes (Crafts, 1939b), *Vitis* phloem shows particularly clearly that the large callus accumulations indicate the passive state of a sieve tube, whereas the conspicuous connecting strands occur during the active state.

The present study raises once more the question of the relation of slime to the pores of the sieve areas. According to Crafts (1939a, 1939b) and others (see review by Esau, 1939), the connecting strands are homogeneous structures and consist of cytoplasm only; if slime is present in the pores, it is forced into them merely by changes (induced by cutting) in the pressure gradient in the sieve tubes. The presence of slime in the large callus masses of those *Vitis* sieve tubes that are just being reactivated (plate 20, A) and the occasional continuity of slime through the sieve plates of the dormant and, consequently, inactive sieve tubes (plate 20, D), do not seem to be related to changes in the pressure gradient and suggest that the slime of two sieve-tube elements is normally continuous across the sieve areas. Moreover, the deep staining of the connecting strands appears to depend on the presence of slime in the pores and not upon the unusual chromaticity of cytoplasm. During dormancy, the thick densely staining slime, within the lumen of the sieve-tube element, is clearly distinguishable from the lightly-staining cytoplasm, and there is no evidence that the chromaticity of the latter is increased because of "premortal" changes (Esau, 1939, p. 389).

Vitis is a good example of a plant in whose aerial parts "pericycle" does not exist as a distinct tissue of independent origin. The fibrous caps that appear on the periphery of the primary vascular bundles are primary-phloem fibers,

or, more specifically, protophloem fibers. Many other dicotyledonous plants were previously shown to have fibers of phloic¹² origin on the outer periphery of the vascular region (see review by Esau, 1943*a*, p. 195-96); recently *Cannabis* (Kundu, 1942), *Linum* (Esau, 1943*b*), *Prunus* (Schneider, 1945), and *Cryptostegia* (Artschwager, 1946) have been added to this group of plants. The ontogenetic relation of the primary fibers to the phloem in *Cryptostegia* is particularly notable because, in a mature stem, these fibers are isolated from the rest of the phloem by parenchyma and appear to be quite independent of the vascular tissues. During stem ontogeny, of course, the sieve tubes associated with the primary fibers "disappear so quickly" that they can be characterized as "evanescent" (Blaser, 1945, p. 140); nevertheless, these sieve tubes and the cells associated with them form an integral part of the phloem system of the plant. Indeed, the protophloem is *the* phloem of the growing region, and in its time and place would seem to be no less essential than the less evanescent phloem in the older parts of the plant. The evanescent nature of certain tissues and structures does not imply a lack of morphologic or physiologic significance. To cite only a few examples: the root hairs; the root-cap cells; the primary endodermis with Casparian strips; many floral parts; cells and tissues involved in sporogenesis and gametogenesis; and, of course, the counterpart of the protophloem in the water-conducting region, the protoxylem—all these have a short existence but are generally regarded as being concerned with some vital part of the life cycle of a plant. The phloem as a whole could be regarded as a transitory tissue, at least in comparison with the xylem, yet all morphologists and physiologists agree that it merits a name of its own. There is accumulating evidence on the phloic origin (in dicotyledons) of the fibers on the outer periphery of the vascular region, and a general adoption of simplified terminology—referring to these fibers as *primary phloem fibers*—seems timely. If the distinction between the primary and secondary tissues is not essential, *phloem fibers* is an adequate term.

The behavior of the secondary phloem fibers in *Vitis* calls attention to still another aspect of the problem of defining fibers. Since De Bary (1884, Chapter II), the loss of protoplasts by fibers has been stressed as a distinguishing characteristic of these elements. In contrast, the "sclerotic cells," that is, the thick-walled forms of parenchyma, are said to remain alive for a long time and partake in the vital function of the plant. De Bary admits that a clear distinction cannot be drawn between the two kinds of cells: in some fibers—and the septate fibers of *Vitis* are given as an example (De Bary, 1884, p. 134)—the functions of the cell cease slowly. Obviously, the septate fibers of *Vitis* (those of the xylem and phloem alike) are concerned with starch storage after they develop their thick lignified walls. In other words, they combine the functions of fibers and parenchyma cells. In certain other plants—for example, the cherry (Schneider, 1945) and guayule (Artschwager, 1943)—the sclerifications of the secondary phloem fibers is delayed until the sieve tubes cease to function. Artschwager (1943, p. 23) specifically mentions that in guayule no difference exists between the phloem-parenchyma cells and the "sclerenchyma initials,

¹² The word *phloic* is adopted here as an adjective derived from the word *phloem*. A precedent in the use of the adjectival form of *phloem* occurs in the well known terms *ectophloic* and *amphiphloic*.

since most of these cells are pointed and eventually thicken and lignify." Similarly, the protophloem fibers are living cells as long as the sieve tubes are intact, becoming mechanical elements only after these sieve tubes are obliterated. Judging from the compactness of the fibrous masses formed in the protophloem, all the cells remaining in the latter after the obliteration of the sieve tubes and companion cells become fibers. In other words, the protophloem may contain no phloem parenchyma as such. (See also Esau, 1943b.) Perhaps fibers sometimes replace phloem parenchyma, either by retaining parenchymatous characteristics until after the sieve tubes lose their function (protophloem fibers of many plants; secondary fibers of cherry and guayule), or by combining the characteristics of parenchyma and fibers (secondary fibers of the grapevine). Recently Kundu (1942, 1944) has reported that even such typical phloem fibers as those of *Cannabis* and *Corchorus* remain alive throughout the life of the plant; and Foster (1944) has called attention to the probable longevity of the protoplasts in sclereids of *Camellia*. Obviously the concepts of fibers, and of sclerenchyma in general, cannot be conceived too narrowly with regard to cell shape and cell-wall structure (phloem fibers are not always lignified), and with respect to cell contents and function.

The absence of the so-called *nacré* walls in the sieve tubes of *Vitis* is worthy of special mention. Transitory thickenings in the differentiating and recently matured sieve tubes are characteristic of many plants (Esau, 1939). Sometimes these thickenings are so prominent that the sieve tube resembles a xylem element in wall thickness (see, for example, Schneider, 1945). In *Vitis*, however, the sieve tubes attain moderate thickness—similar to that of the phloem-parenchyma cells—and retain it till they are cast off by cork development.

SUMMARY

The phloem of *Vitis vinifera* was studied through axis samples collected in a California vineyard at regular intervals during two years. The phloem of the canes (one-year-old branches) was investigated in particular detail, but that of the trunks and roots also was examined.

In addition to the usual iodine-aniline blue technique for studying phloem, a stain combination of Bismark brown, iodine green, and resorcin blue was employed, the staining being followed by the mounting of sections in Karo sirup. The latter stain combination differentiated very clearly between the callose and the sieve-tube slime and produced semipermanent mounts useful for survey work and for detailed studies of the sieve tubes.

The primary phloem of the shoots can be divided into protophloem and metaphloem. In the former, the sieve tubes are obliterated during shoot development and the remaining cells differentiate into septate fibers. The metaphloem has no fibers. The protophloem is cut off by cork at the end of the season and the metaphloem becomes functionless.

The secondary phloem is divided into blocks by broad parenchyma rays. Within each block tangential bands of fibers alternate with bands containing sieve tubes, companion cells, and phloem parenchyma. Each season several fiber bands and several sieve-tube bands are produced by the cambium. Each seasonal increment begins and ends with a sieve-tube band. The phloem formed at the end of the season is composed of very narrow cells and contrasts rather

sharply with the wide-celled phloem that follows it the next season. Hence it is possible to distinguish between the annual increments.

Each annual increment of secondary phloem is retained on the plant for at least two seasons; then most of it is cut off by the cork. At the end of the season, younger axes usually have one increment of phloem and a small part of another, older increment; older axes may accumulate two, three, and even four increments.

In the aerial plant parts a given phloem increment functions during the season when it is formed; it is dormant during the winter, is reactivated in the spring, and functions for another season. If it is not cut off by the cork at the end of the second season, it may become reactivated two or even more times. Root phloem does not appear to become dormant.

Since the phloem is sloughed off rather regularly after it ceases to function, only a relatively small amount of functionless (nonconducting) phloem accumulates on the stems just beneath the cork. This amount is usually very small in young stems but may be larger in trunks. Roots appear to have irregular formation of cork and a consequent accumulation of considerable amounts of functionless phloem.

The reactivation of the phloem precedes that of the cambium and appears to show no comparable initial localization in relation to bud growth.

The phloem activity during a given season may be summed up as follows. The reactivation of the phloem that was formed during the preceding season begins about two weeks before the cambial divisions start. This phloem is in full function before much of the new phloem has been produced. When the latter is fully formed, the old phloem disorganizes and is cut off by the cork. The new phloem functions until dormancy sets in after leaf fall.

In addition to the topics just summed up, this paper considers in some detail: the comparative structure of the active, dormant, and functionless phloem; the ontogeny and histology of the phloem; the activity of the vascular cambium; and the formation of cork.

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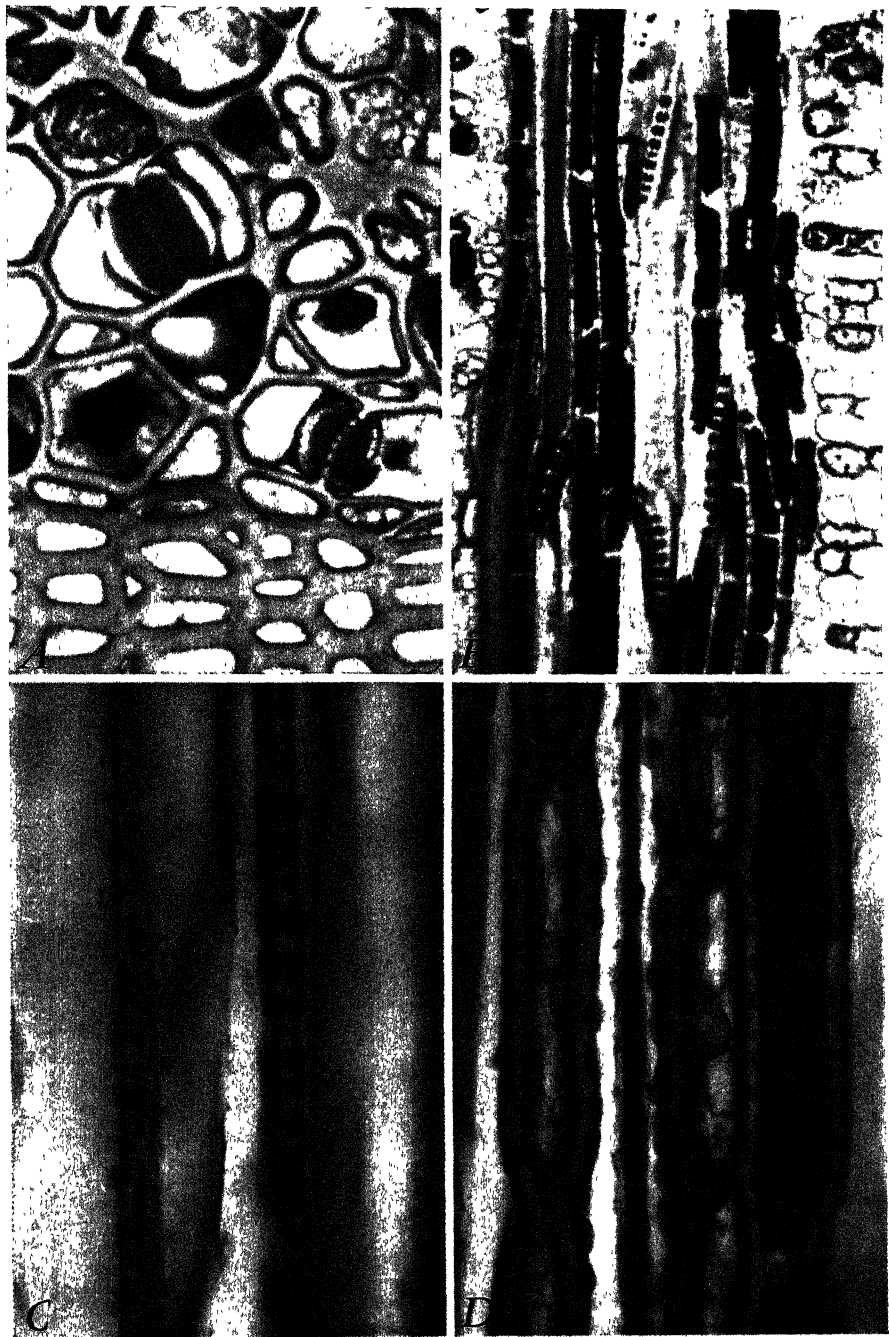
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Plate 1. Illustration of the results of staining of phloem with the following combination: Bismark brown, iodine green, and resorcin blue. The callose is stained blue. (The callose in *C* and *D* appears more green than it usually does in the slides.) *A*, Transverse section through partly reactivated phloem collected April 7, 1945. Connecting strands are visible in the lower callus mass. (Compare with plate 10, *A*.) *B*, Longitudinal section through the 1944 phloem that has just ceased to function. Collected July 3, 1945. Definitive callus occurs on all sieve areas. *C* and *D*, Longitudinal sections through dormant phloem collected December 3, 1946. The most prominent masses of callus in *C* are on a wall between two sieve-tube elements. In *D* the callus occurs on the walls between sieve tubes and phloem-parenchyma cells; it has been formed only on the sieve-tube sides of the walls. (*A* $\times 320$; *B* $\times 200$; *C* and *D* $\times 800$.)



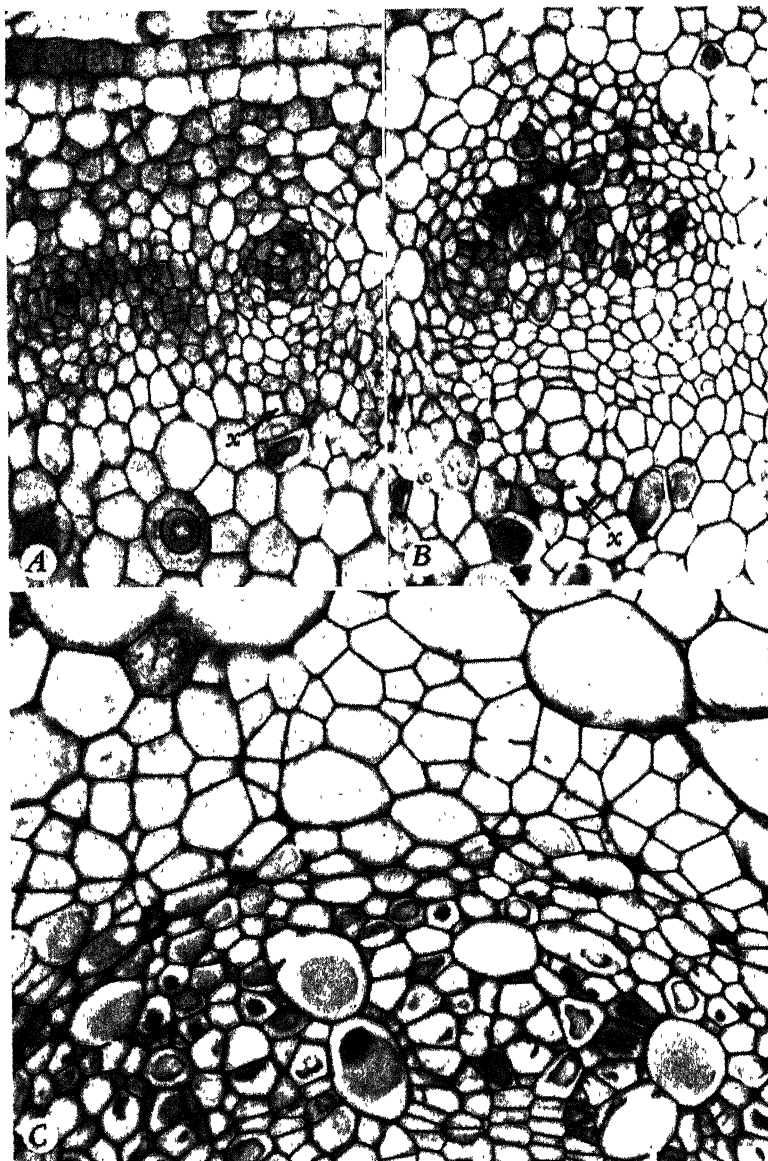


Plate 2. Transverse sections through a shoot illustrating stages in the differentiation of the primary phloem. *A*, Two procambial bundles: one, to the left, with the first sieve-tube (thick-walled cell); the other, to the right, with several sieve tubes and one xylem element (*x*). *B*, Young vascular bundle still lacking cambium. Many sieve tubes have been obliterated in the outermost part of the bundle; their remnants look like darkly stained wall thickenings. A radial row of xylem elements occurs at *x*. *C*, Phloem of a bundle in which primary growth was nearing its end. The regions from the top downward are: cortex (largest cells); nonfunctioning phloem (protophloem) in which the sieve tubes are crushed and the future fibers enlarged; functioning phloem (metaphloem). (All $\times 500$.)



Plate 3. Transverse sections through a seedling stem showing an earlier (A) and a later (B) stage in the initiation of cork. In both sections from the top downward are: cortex; primary-phloem fibers (*f*); phloem-parenchyma cells dividing to form cork (*c*); functioning phloem (mostly metaphloem); cambium; xylem. (Both $\times 290$.)

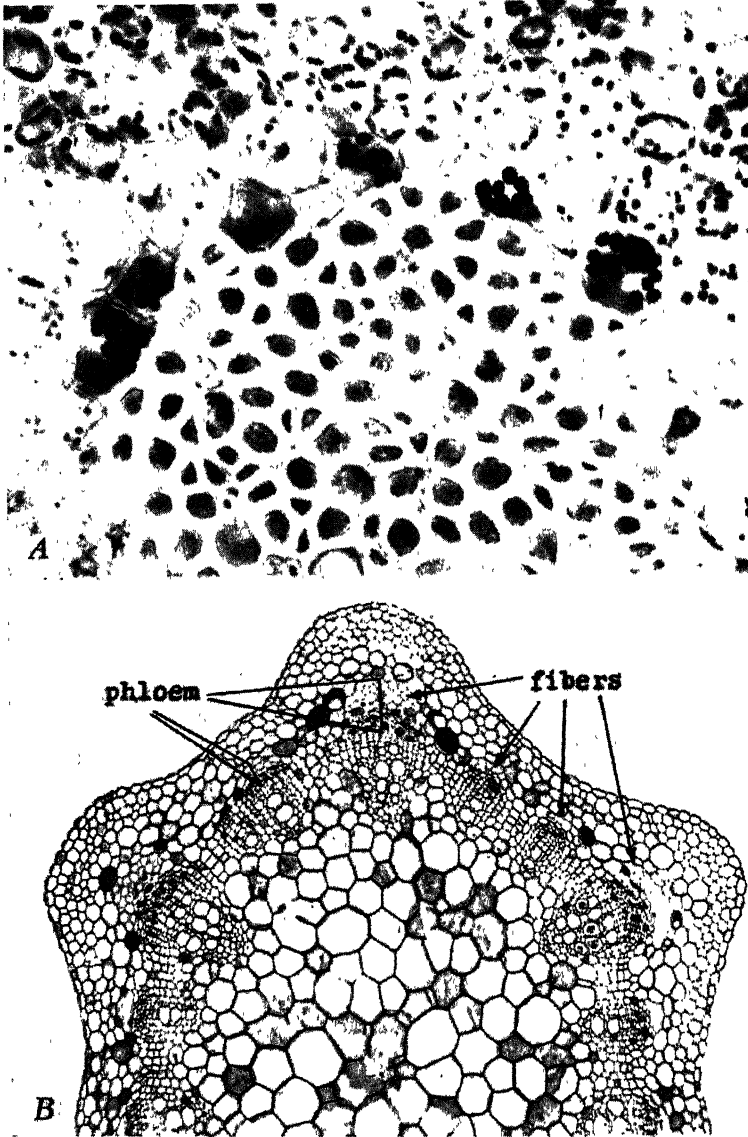


Plate 4. *A*, Transverse section through primary-phloem fibers (below) and cortical cells (above) from a new shoot. The black bodies in the cortical cells are starch grains stained with iodine. The cortical cells with the large starch grains next to the fibers constitute the starch sheath. *B*, Transverse section through a seedling stem in which primary growth was completed. From outside in the tissues are: epidermis; cortex; primary phloem, including the fibers; cambium; xylem; and pith. (*A* $\times 290$; *B* $\times 90$.)

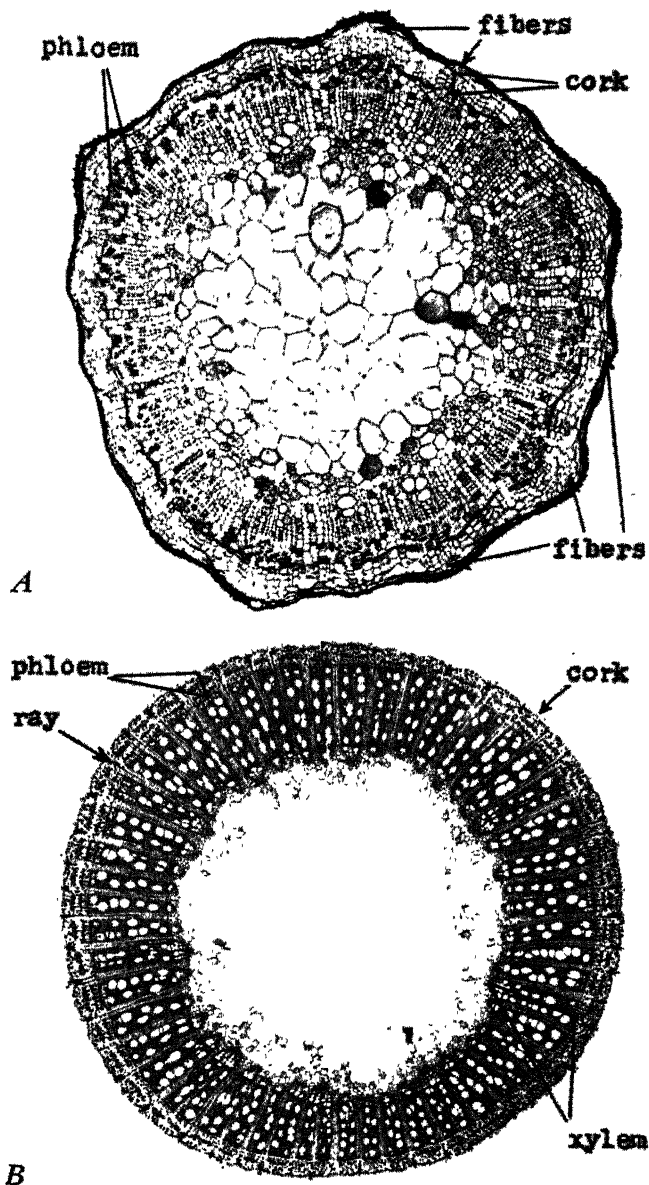


Plate 5. Transverse sections through a seedling stem (*A*) and a mature cane (a one-year-old branch) (*B*). The stem in *A* shows early secondary growth: some secondary xylem and phloem are present and cork occurs beneath the primary-phloem fibers. The latter are still intact, but the cortex (on the outside of the cork) is collapsed. In *B* the epidermis, the cortex and the primary-phloem fibers have been sloughed off. Only a small part of the xylem (next to the remnants of the pith) is primary; the rest is secondary. (*A* $\times 50$; *B* $\times 10$.)

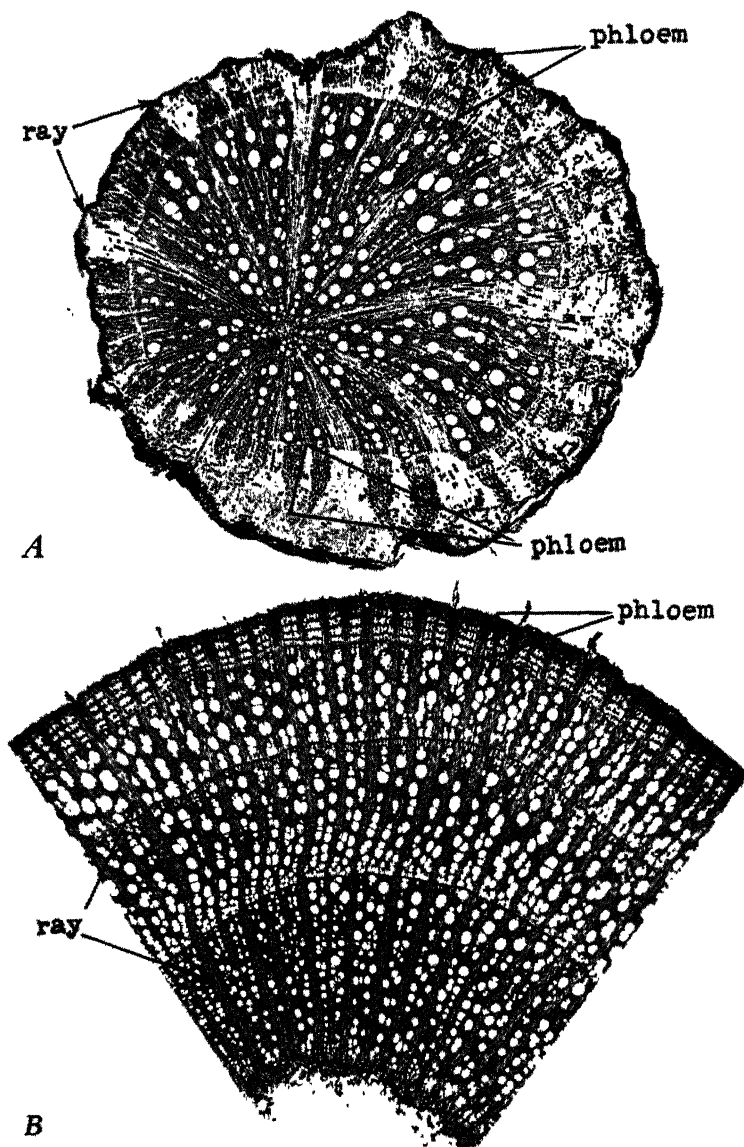


Plate 6. Transverse sections (*A*) through a four-year-old root and (*B*) through a part of a three-year-old branch (arm). The lack of distinctness of the annual rings, the great width of some of the rays, and the great thickness of the bark distinguish the root from the branch. (*A* $\times 9$; *B* $\times 8$.)



Plate 7. Transverse (A) and tangential longitudinal (B) sections of secondary phloem from a cane in second year of growth. The cane was collected July 3, 1945, just before cork was to be formed. In A the regions from left to right are: 1944 cork; 1944 phloem ending with the late phloem at *a*; 1945 phloem. All phloem to the left of *a* is functionless. The section in B was taken from the functioning phloem. Ray cells occur above and below in the photograph. Between these are several complete sieve-tube elements, with sieve plates (*p*) in sectional views, and many phloem-parenchyma cells. (The material stained black in the phloem-parenchyma and ray cells is tannin.) Details are: *a*, late phloem of 1944; *b*, crystal-containing layer; *f*, secondary-phloem fibers; *p*, sieve plate. (A $\times 90$; B $\times 150$.)

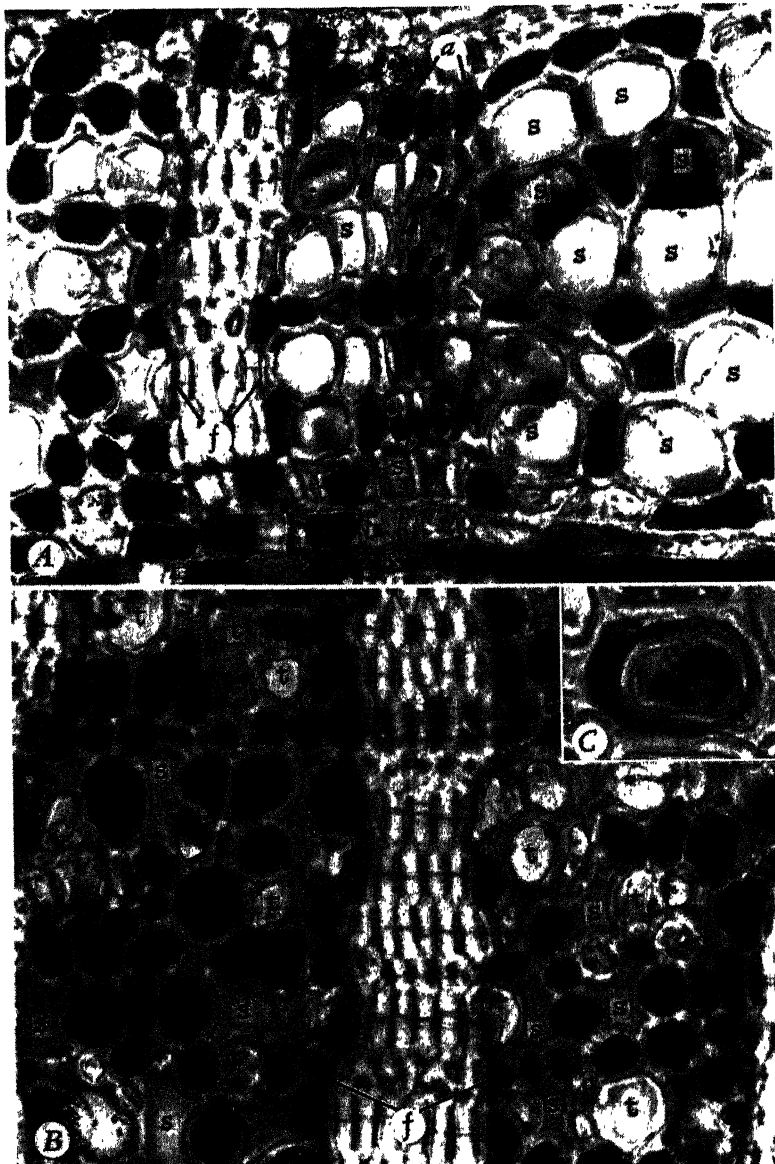


Plate 8. Transverse sections of functioning (A) and nonfunctioning (B) phloem from the trunk of Ohanez variety, collected June 25, 1945. To the right in A is the 1945 phloem. At *a* is the small-celled late phloem of 1944. The sieve tubes of the 1944 phloem in B either contain tyloses (*t*) or are partly crushed (*s*). At C is a single sieve tube with a tylose in transverse section. The black bodies within the tylose are starch grains; the black substance between the tylose and the sieve-tube wall is the residue of sieve-tube contents. Details are: *f*, secondary-phloem fibers; *s*, sieve tube; *t*, tylose. (A and B $\times 290$; C, $\times 750$.)



Plate 9. Radial longitudinal sections of the functioning (A) and nonfunctioning (B) phloem from the trunk of Ohanez variety, collected June 25, 1945. The youngest phloem in A is at the top of the photograph. Several complete sieve-tube elements with the sieve plates (p) in face view occur in A. In B the sieve-tube elements are indistinguishable because they are filled with tyloses. Details are: f, secondary-phloem fibers; p, sieve plate. (Both $\times 150$.)

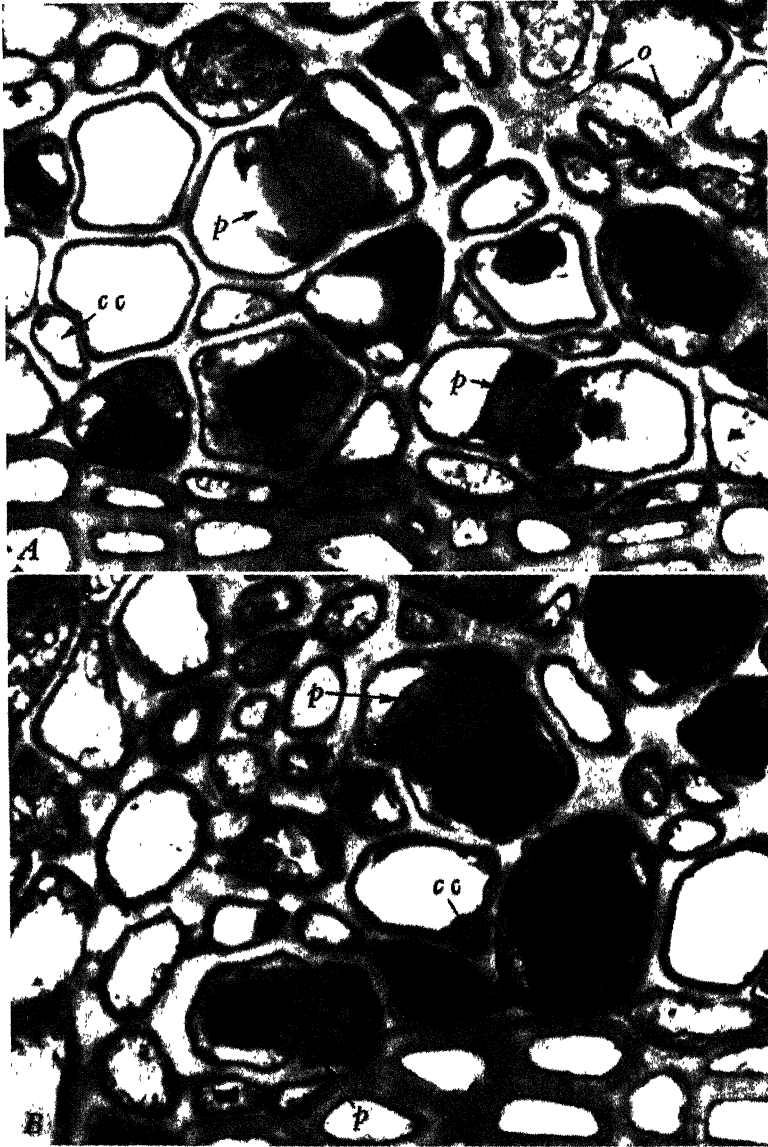


Plate 10. Transverse sections through the peripheral portion of reactivating phloem from a cane collected April 7, 1945. Two calloused sieve plates (*p*) occur in each photograph. The connecting strands are barely perceptible in the upper callus mass in *A*; they are conspicuous in the lower plate in *A* and in both plates in *B*. Slime accumulations are associated with the callus in *B*. Details are: *cc*, companion cell; *o*, obliterated sieve tubes; *p*, sieve plate. (Both $\times 750$.)

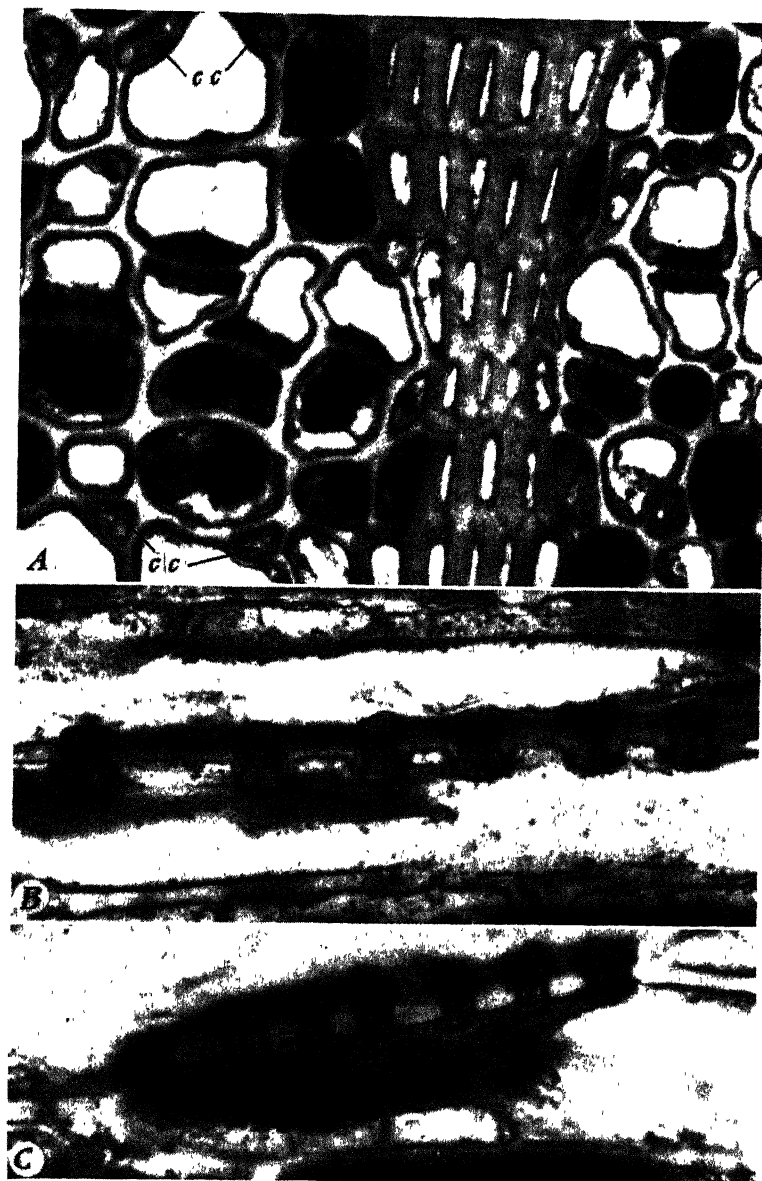


Plate 11. Transverse (A) and tangential longitudinal (B and C) sections through the reactivating phloem of a cane collected April 7, 1945. The section in A occurred closer to the cambium than the ones in plate 10. Six sieve plates, with prominent connecting strands, occur in A. Companion cells are at cc. The compound sieve plates in the longitudinal (B) and end (C) walls show a callus mass with connecting strands in each sieve area. The darkly stained material near the sieve areas in all photographs is slime. (All $\times 750$.)

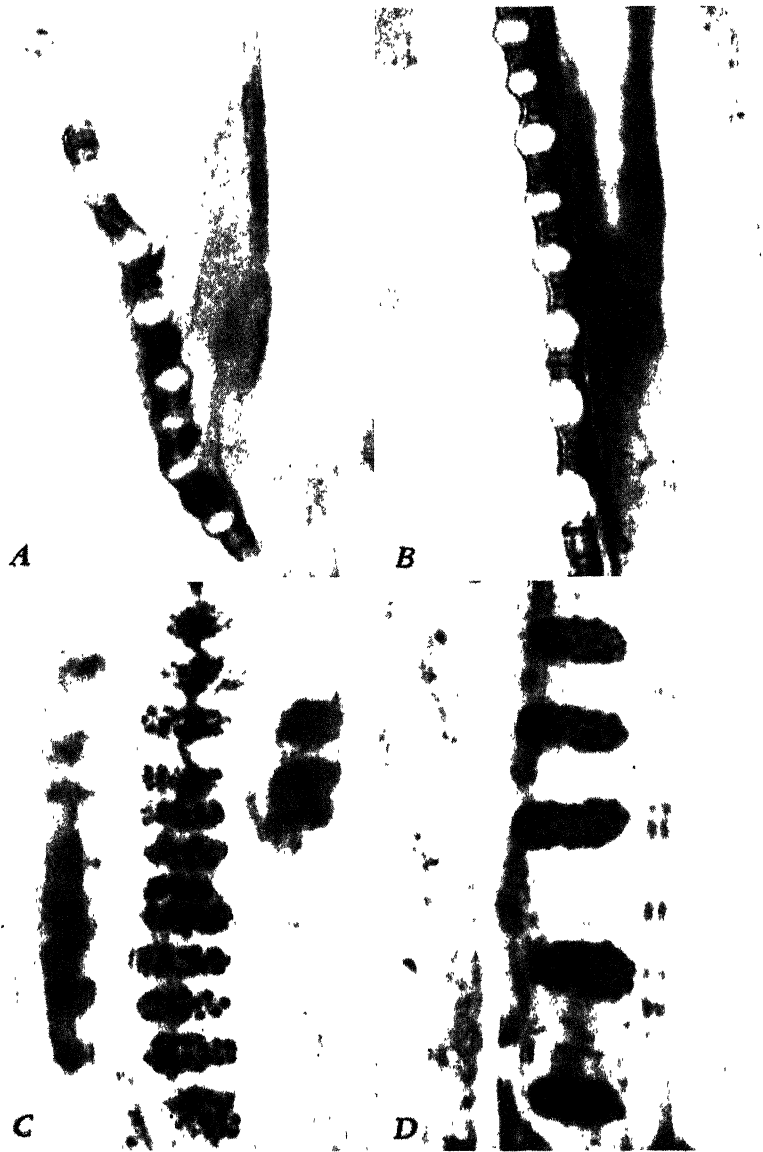


Plate 12. Sectional (*A* and *B*) and face (*C* and *D*) views of sieve plates of newly differentiated phloem. Some of the connecting strands in *A* and *B* show knobs at the surface of the sieve areas. The darkly stained material in *A* and *B* is slime; the lightly stained material in *A*, cytoplasm. The latter is connected to the sieve plate and contains starch grains, circular in outline. *C*, Sieve plate on an end wall with sharply outlined callus cylinders in the sieve areas. In *D*, showing a sieve plate in a longitudinal wall, the callus cylinders and the connecting strands together appear as black dots. The entire sieve areas are rather dark because they are completely covered with callus. (All $\times 750$.)



Plate 13. Tangential longitudinal sections from active (*A* and *D*) and dormant (*B* and *C*) phloem. *A* and *D* were collected May 30, 1945; *B* and *C*, March 10, 1945. *A* shows several sieve plates with the associated slime in sectional view; *B*, a compound sieve plate in section; *C*, sieve areas of type B in face view (above) and sectional view (below); *D*, above, sieve areas of type B in face view ($A \times 290$; $B-D \times 750$.)

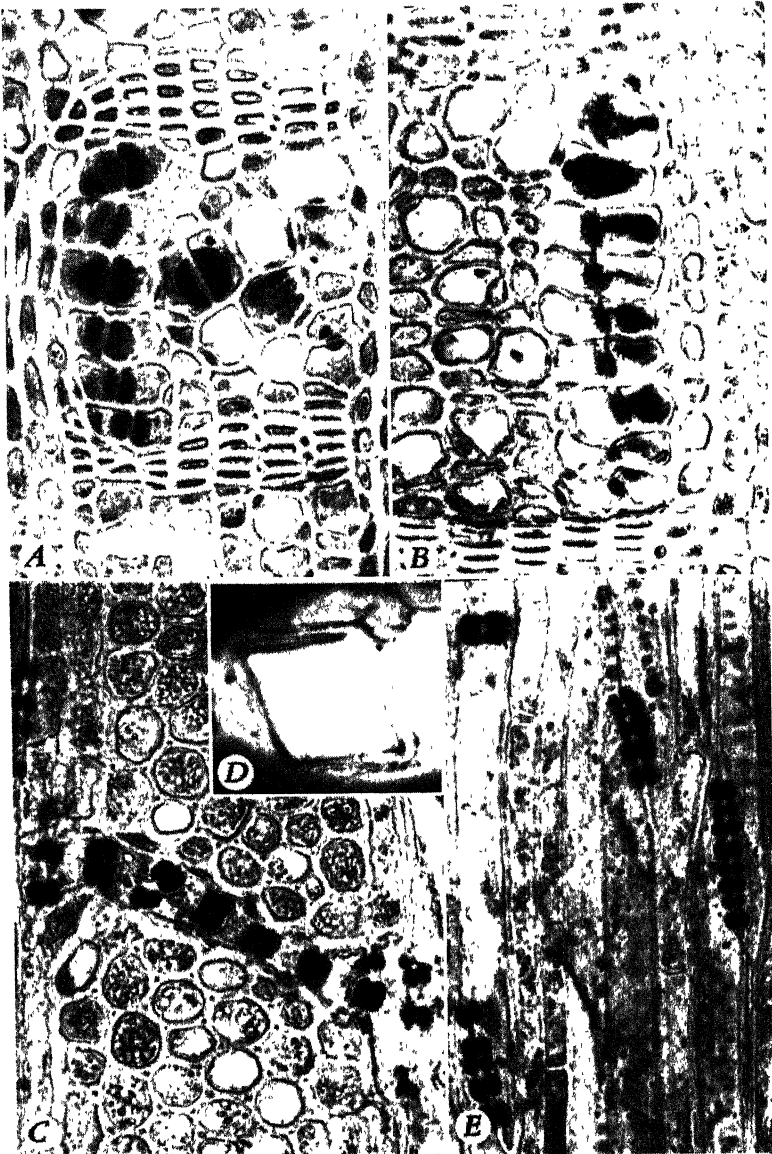


Plate 14. Transverse (*A* and *B*) and tangential longitudinal (*C* and *E*) sections of 1944 phloem collected March 10, 1945 (*A*, *C*, and *E*) and April 23, 1945 (*B*). *A*, *C*, and *E*, Dormant phloem with massive callus on the sieve plates. In *C* a series of small sieve-tube elements traverse a ray. *B* shows signs of resumption of active state: the callus masses are thin, and slime is visible near the sieve plates. *D*, Part of a single ray cell with a crystal imbedded in wall material. (*A*–*C* and *E* $\times 290$; *D* $\times 1200$.)

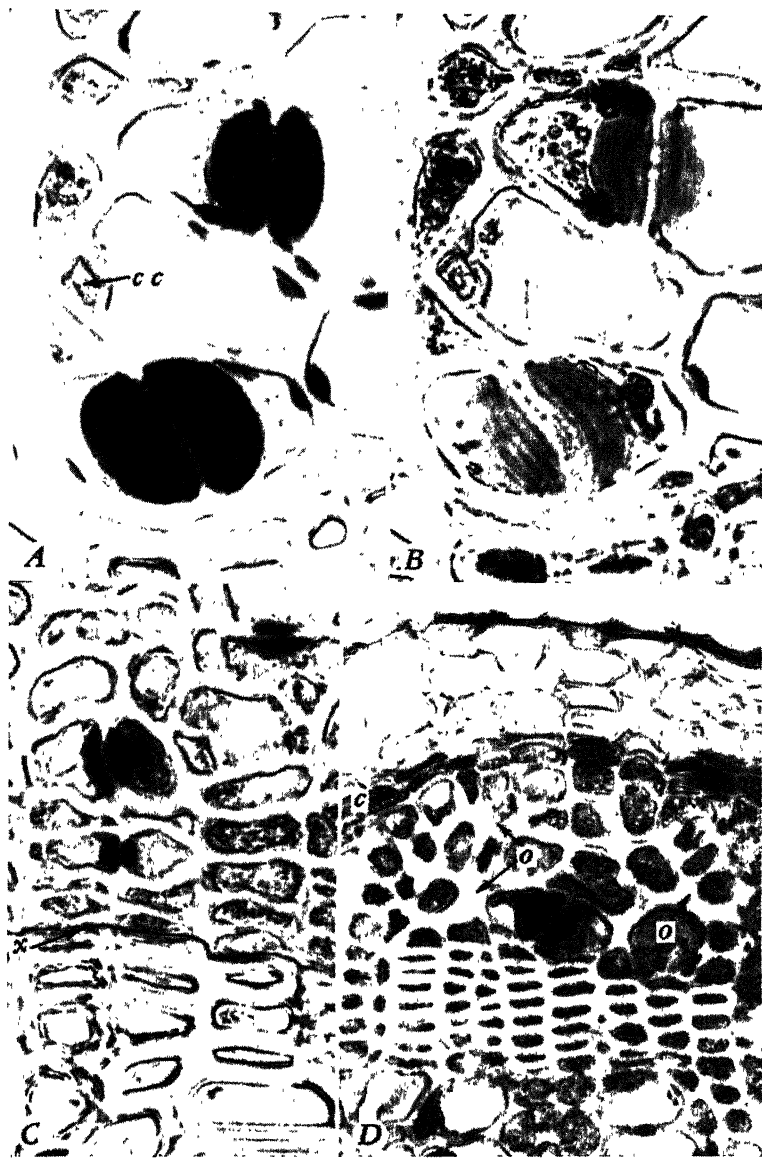


Plate 15. Transverse sections through dormant (*A-C*) and nonfunctioning (*D*) 1944 phloem collected March 10, 1945 (*A-C*) and April 23, 1945 (*D*). *A* and *B* are photographs of the same section, one taken with Wratten filter F (red), the other with Wratten filter B (green) and a much reduced opening in the substage-condenser diaphragm. In both sections the callus was stained blue. *A* shows callus cylinders traversing the cellulose wall of the sieve plate. *B* illustrates the layering in the callus. *C* shows the late 1944 phloem, cambium, and xylem. The latter is sharply delimited from the cambium at *x*. In *D*, the four to five cells on the upper surface are cork cells (above *c*). Beneath the cork most of the sieve tubes have been obliterated (*o*). (*A-C* $\times 750$; *D* $\times 290$.)

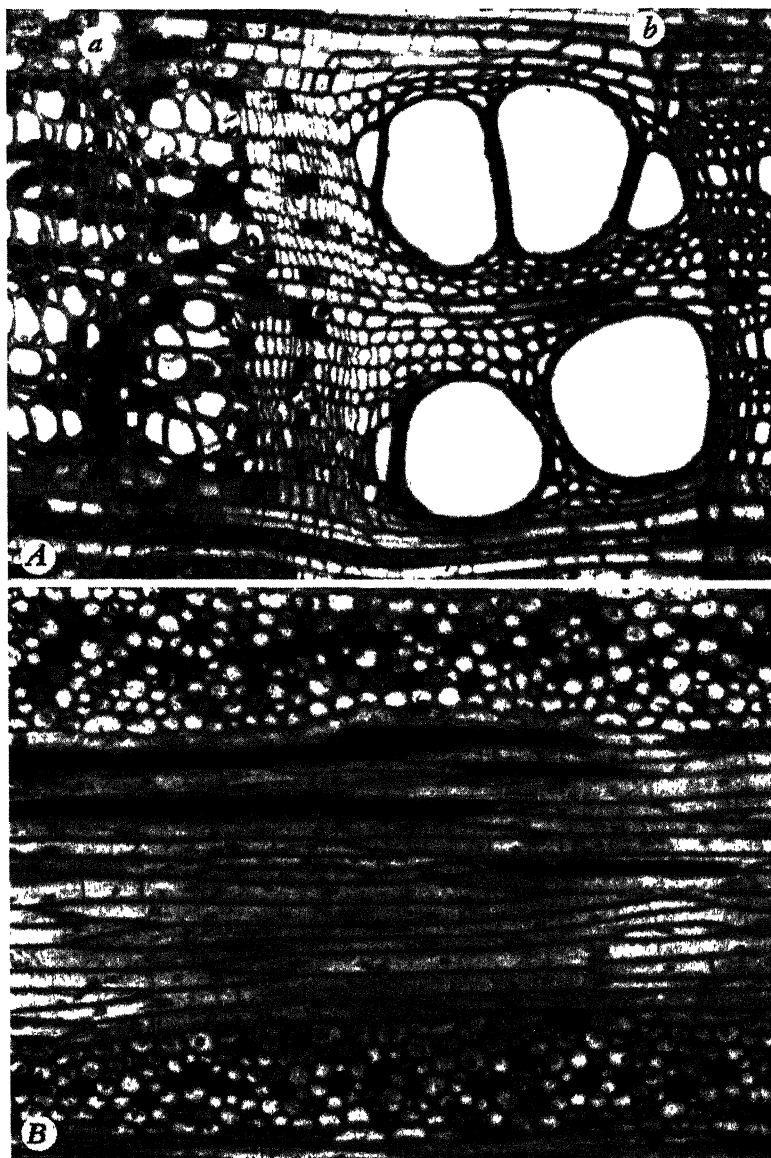


Plate 16. Transverse (A) and tangential longitudinal (B) sections through the cambium and adjacent tissues of a cane in second year of growth, collected May 30, 1945. A shows at *a* the late 1944 phloem, at *b* the end of 1944 xylem. Between the 1945 phloem (to the right from *a*) and the 1945 xylem (to the left from *b*) lies the fascicular-cambium region. B shows rays above and below, and cambial initials and partly differentiated phloem (with tannin-containing cells) between the rays. (Both $\times 140$.)

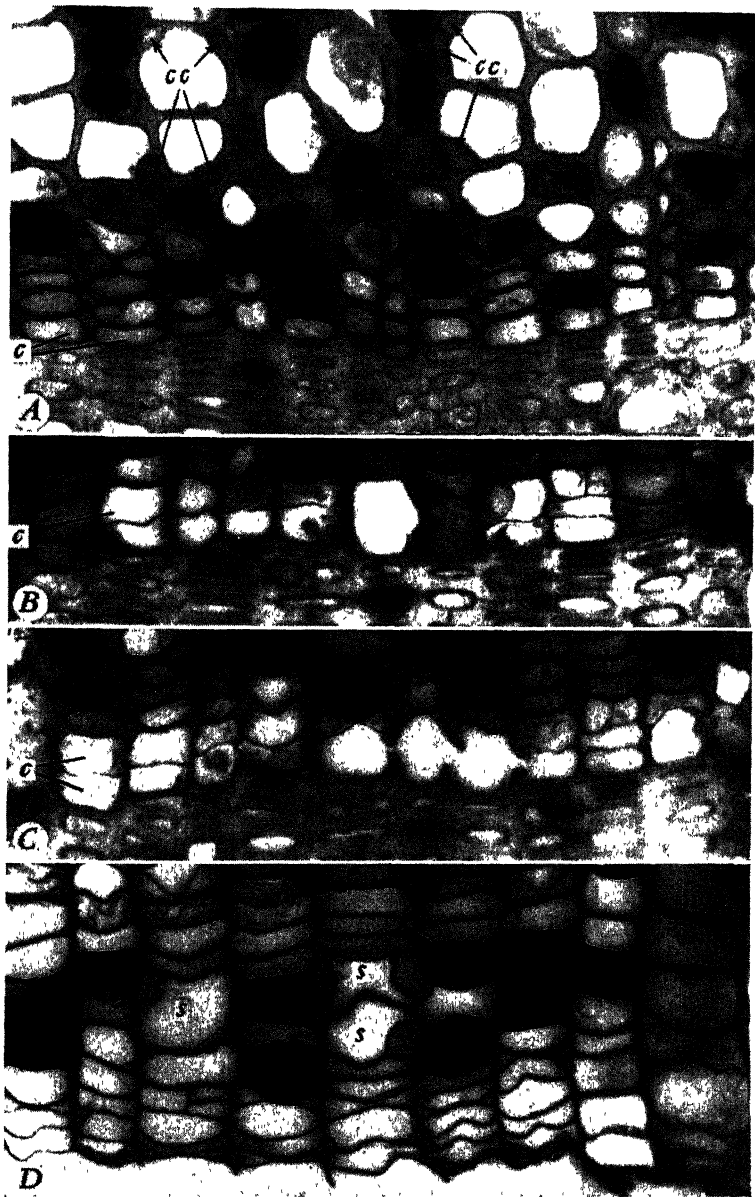


Plate 17. Transverse sections through the cambial region during dormancy (*A*), at the beginning of cambial activity (*B* and *C*), and at the end of this activity (*D*). Sections are from canes in second year of growth collected April 7, 1945 (*A-C*) and July 3, 1945 (*D*). In *A-C* the cambium is at *c* and the xylem below it. Companion cells are at *cc* in *A*. In *D* the cambium occurs at the lower edge of the section (the bark separated from the wood along this edge) and above it are some partly differentiated sieve tubes (*s*). (All $\times 450$.)

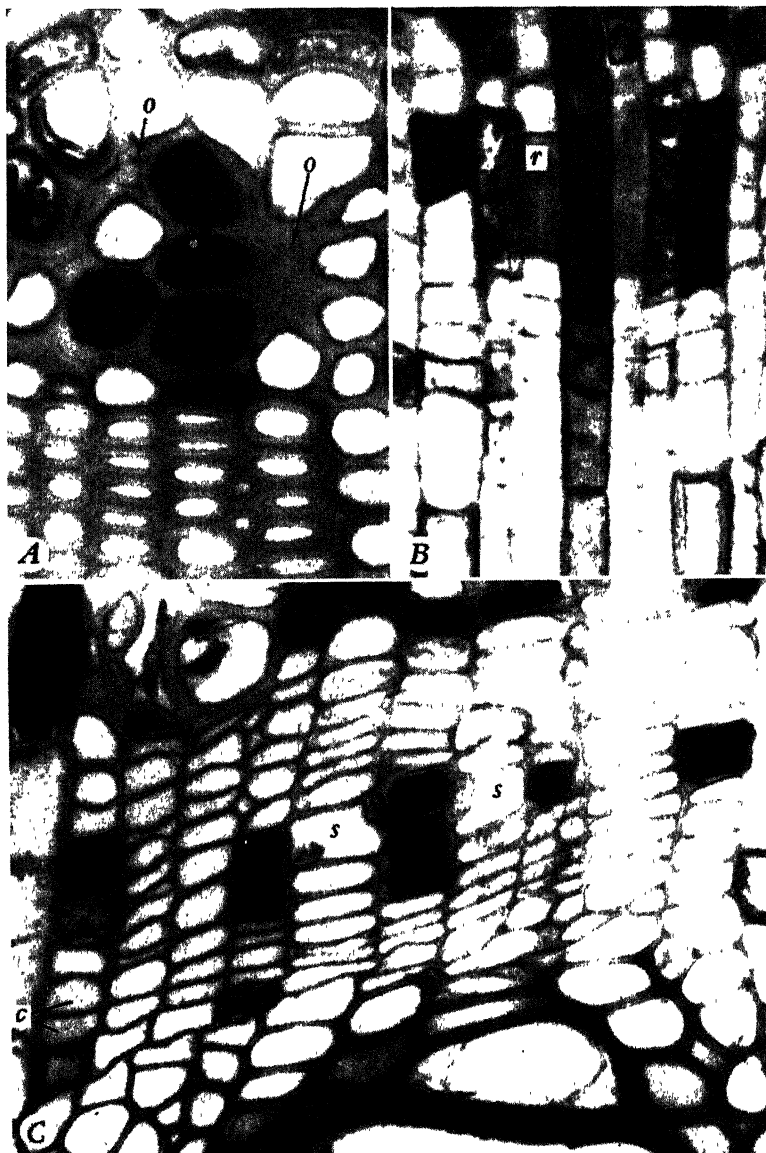


Plate 18. Transverse sections through the regions of: *A*, sieve-tube obliteration; *B*, interfascicular cambium; and *C*, fascicular cambium. All from a cane in the second year of growth collected May 30, 1945. (The same cane was used for plate 16.) In *A* the crushed sieve tubes appear at *o*. In *B* the cells functioning as ray initials in the median column of cells contain tannins; raphides (*r*) appear in cells that lie very close to the dividing ones. In *C* the cambium proper is at *c*. Tannin-containing cells occur on both sides of the cambium; some differentiating sieve tubes (*s*) are above it. (*A* and *C* $\times 450$; *B* $\times 290$.)

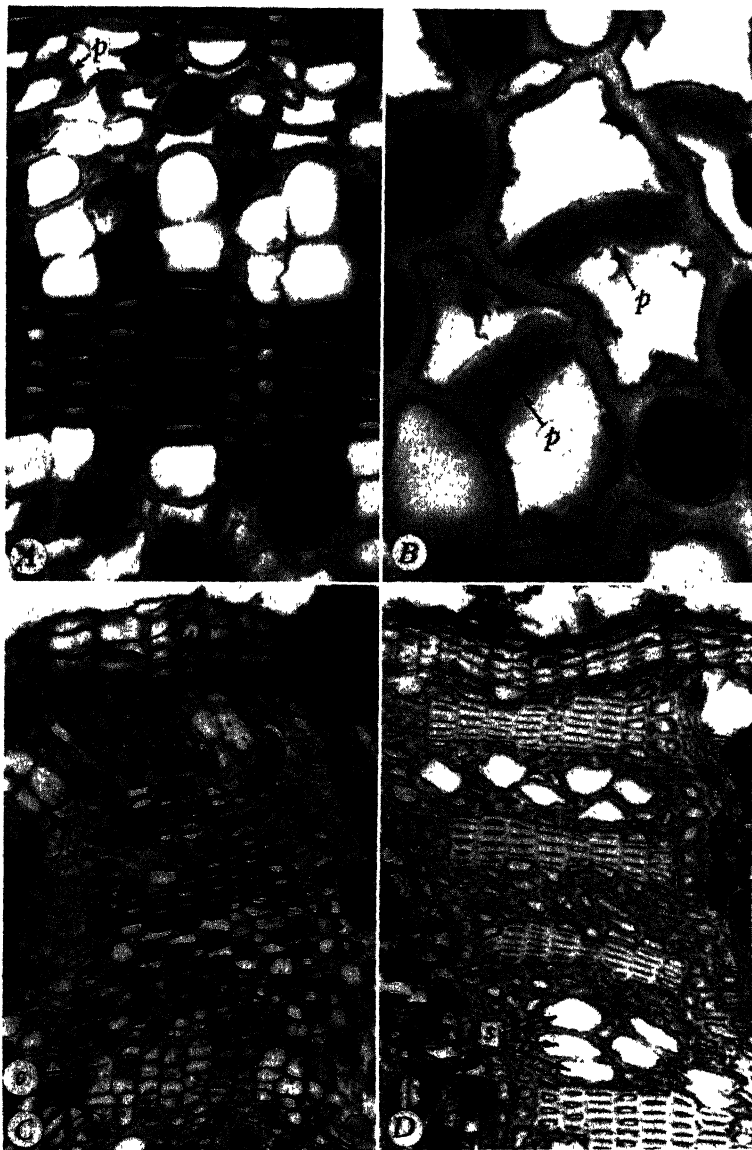


Plate 19. Transverse sections of phloem from trunks (A and D) and canes (B and C) collected July 3, 1945 (A-C) and April 17, 1945 (D). A, Initiation of cork cambium. Parenchyma cells above the fibers have enlarged and divided. The sieve tubes are partly crushed above the divided cells. Sieve plates with definitive callus at *p*. B, Functionless 1944 phloem with definitive callus on the sieve plates (*p*). In C the 1944 functionless phloem (above) has been separated from the functioning 1945 phloem (below) by cork (*c*). The 1944 cork occurs along the upper edge of the section. D, Dead phloem of 1943 that was cut off by cork formation in 1944. Empty sieve tubes appear as *s*. (A $\times 290$; B $\times 750$; C and D $\times 150$.)

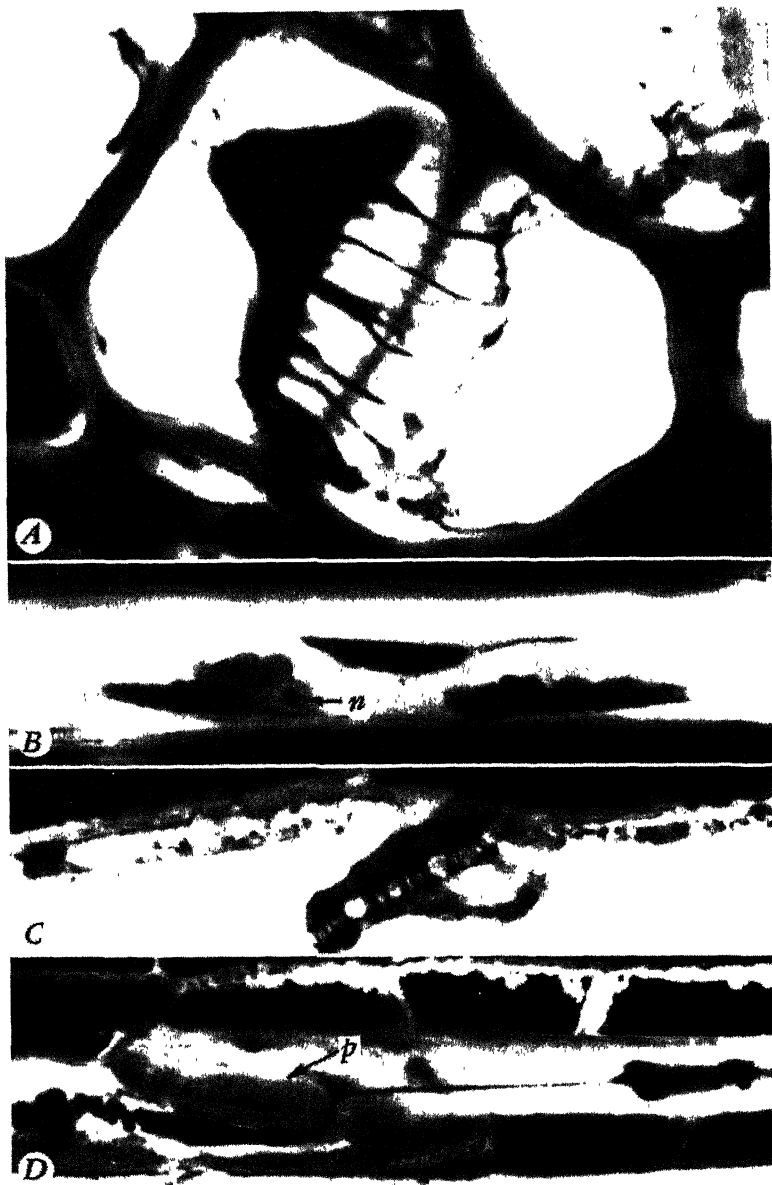


Plate 20. Transverse (A) and longitudinal (B-D) sections of sieve tubes showing different states of metamorphosis of sieve-tube slime. The material was collected on the following dates in 1945: A, April 25; B, May 30; C, September 13; D, December 8. In the center of the picture in A, the slime projects into the callus mass of the sieve plate from a reactivating sieve tube. B, Slime bodies and a nucleus (*n*) in an immature sieve-tube element. C, Part of a sieve tube from functioning phloem. The slime on the sieve plate has projections passing through the plate. D, Part of a sieve tube from dormant phloem. The sieve plate (*p*) is covered with callus, but in one place a strand of slime passes through it and connects the slimy accumulations on the two sides of the plate. (A $\times 1200$; B and C $\times 750$; D $\times 290$.)

H I L G A R D I A

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SAMPLE MANIPULATION AND APPARATUS USEFUL IN ESTIMATING SURFACE AND PENETRATION RESIDUES OF DDT IN STUDIES WITH LEAVES AND FRUITS^{1, 2}

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INTRODUCTION

THE CONSEQUENCES of DDT applied in the field have been intensively investigated at the University of California Citrus Experiment Station since 1943. Early in this investigation certain instances disclosed that DDT, as it was applied in the field in various experimental formulations, actually penetrated the fruit and leaf tissues. This indicated potential consumer hazards. Therefore, a detailed semiquantitative examination of surface residues and of the extent and degree of penetration resulting from such applications was undertaken.

Materials examined in this program included fruit, and sometimes leaves, from mature apple, avocado, citrus, olive, peach, pear, and plum trees. At the present time a report of the analytical techniques developed empirically from the handling of many thousands of these samples, and a detailed description of some of the apparatus used may be of benefit to other investigators.

Carter and Hubanks (1946)⁴ recently presented a brief discussion of apparent "recoveries," by analytical methods, of DDT added to dried plant material, including apples. Their results agree with those obtained by the techniques developed during the present investigation. Moreover, Wichmann *et al.* (1946) recently have thoroughly discussed the application of three methods for the quantitative determination of DDT as spray residues on fresh fruit, particularly apples and pears. Certain points of similarity between the techniques discussed by Wichmann *et al.*, and those discussed in the present paper will be noted.

It must be emphasized that certain of the manipulative procedures described in this report are only semiquantitative in nature. This is particularly

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⁴ See "Literature Cited" at the end of this paper for complete data on citations, referred to in the text by author and date of publication.

true of the treatments designed for the recovery of penetrated DDT. A careful check of all such semiquantitative procedures indicates 75 to 90 per cent recovery of penetrated DDT. Quantitative recovery data for the most important techniques have been included in the appropriate sections.

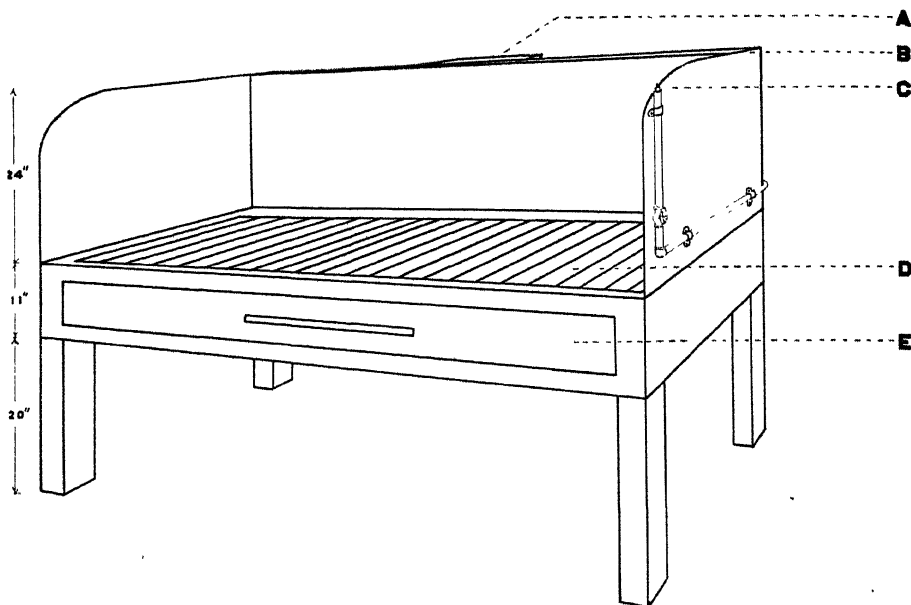


Fig. 1.—Downdraft hood designed especially to handle benzene vapors during stripping operations in DDT surface-residue and penetration studies. *A*, vent; *B*, false back; *C*, compressed-air pipe; *D*, slotted floor; *E*, plenum chamber. None of the dimensions shown is critical, as they were determined by convenience only.

Heavier-than-air vapors are drawn through the slotted floor (*D*) into the plenum chamber (*E*) below, up through the false back (*B*), and are then exhausted out of doors through the vent (*A*). An explosion-proof motor and an efficient fan are mounted in the vent (*A*). The slotted openings in the floor (*D*) of the hood are parallel to the air stream to avoid turbulence foci. A large shallow pan placed on the floor of the plenum chamber (*E*) prevents spilled materials from attacking the plywood flooring. The front panel of this chamber (*E*) is removable to allow easy access for cleaning and for recovering dropped objects. The pipe (*C*) is connected to the laboratory compressed-air supply, and is fitted as shown, with a needle valve and a tubing nipple. A graduated cylinder, thumb-controlled wash bottle (fig. 2) is connected to this nipple by means of a convenient length of rubber tubing threaded through an ordinary screen-door spring. This is to allow for flexibility of manipulation and freedom of interference and yet eliminate danger of collapsing or kinking of the tubing. (Drawing by Miss Athalie Thomas.)

MATERIALS AND METHODS

Penetrated DDT is usually expressed in terms of parts per million by weight. Surface DDT may also be expressed in terms of parts per million, but it is usually best expressed in terms of micrograms of DDT per square centimeter of surface. In the present studies, leaf areas were measured by

means of a photoelectric arealimeter;⁵ fruit surfaces were estimated by means of numerical tables of spheroidal surfaces (Turrell, 1946), in which measurements of the major and minor axes are translated into the areas involved.

The surface residual or the penetrated DDT was removed from the samples by solution in benzene (benzol). Benzene was selected for this purpose because of its high solvent power for DDT (Gunther, 1945a), its low cost, its ready availability in large quantities, and its poor solvent power for most inorganic chlorides and for such constituents of fruits and leaves as sugars, many pigments, and many fatty and waxy materials.

Benzene is a very toxic substance, however, and its vapors can be dangerous if inhaled over a long period. Experience has shown that the ordinary updraft hood will not adequately handle the dense benzene vapors. For example, in the fruit-stripping operations described later it quickly became apparent that the normal turbulence at the front of the hood did not provide for sufficient mixing to protect the operator from the benzene vapors. This was true even when the hood window was nearly closed and only the arms of the operator extended into the working space of the hood. One operator became gravely ill after working intermittently under these conditions over a period of a few months. The solution for this problem was a downdraft, slotted-floor hood (fig. 1).⁶

Leaf Studies

Sampling.⁷ Satisfactorily consistent analytical agreement between replicates of field samples of citrus leaves involved the use of two operators. Six trees in the plot were selected at random, the only requirements being that they must not be the trees treated either first or last from the tank of spray or dust material, and that they must not be manifestly atypical trees.

The first tree was circled clockwise by the first operator, and 5 average-sized mature leaves were selected at random from a foot-wide belt at chest height from each quadrant. Ordinarily, leaves from the same cycle of growth were chosen. This was done because of possible unknown effects of the nature of the leaf surface upon initial deposit, subsequent rate of penetration, and rate of weathering. For precise work, the leaves were held with a pair of long specimen forceps while the stem was clipped with a pair of fruit-picking shears. In this way, loss or transfer of deposit by finger contact was avoided. For most routine purposes, however, picking by fingers was done to save time.

The leaves were placed in a wide-mouth 2-quart Mason jar suspended from the neck of the operator by a rope harness. After the tree had been completely circled, the twentieth leaf was picked at the starting point. In this manner, the first operator circled the first, third, and fifth trees, and thereby collected 60 leaves. Meanwhile, the second operator circled counterclockwise the second,

⁵ Apparatus designed and constructed by the writer, with the assistance of C. W. Barnhardt. For details, see figures 3 to 7, with text description.

⁶ Apparatus designed and constructed by the writer, with the assistance of H. U. Meyer and C. R. Shafer.

⁷ Based in part on a statistical analysis of valid sample size and technique of sampling as conducted in connection with another project (A. M. Boyce and J. F. Kagy. Unpublished manuscript, 1939). The double-operator and reverse-circling modifications have proven their worth in helping minimize through randomization the so-called "psychological factors" involved in field sampling of citrus leaves.

fourth, and sixth trees, and similarly collected 60 leaves. At this point the two operators exchanged jars. Trees two, four, and six were then sampled by the first operator with his clockwise circling, and trees one, three, and five by the second operator with his counterclockwise circling.

Through this complicated procedure, a highly desirable divorce from the personal factor was secured in the selection of leaves. Thus, each sample of the 120-leaf duplicate pair was a composite from six random trees, picked in a consistent manner by two operators working always from opposite directions and contributing the same number of equivalent specimens to both samples. Further randomization was obtained by having the operators work alternate trees, then recycle to pick up the skipped trees.

The jar containing these 120 leaves was capped with waxed paper and a Kerr lid assembly. It was then stored in the shade while awaiting transport back to the laboratory. Samples for later use were placed under refrigeration in the laboratory until needed. Most of the leaves studied can be held at 40° F for 2 to 3 weeks before serious decomposition sets in.

Manipulation for Residue Analyses. Exactly 120 average-sized (about 25 square centimeters of single surface area per leaf) mature leaves were collected from the treated trees at the cessation of drip or runoff, as described above. After approximately 24 hours (Gunther *et al.*, 1946), the total surface area of the sample was determined photoelectrically. The leaves were replaced in the jar with 150 ml of benzene, and the jar was capped with a Kerr lid assembly over four layers of waxed paper. The jar was then vigorously shaken manually, 20 times vertically and 20 times horizontally, with rotation; it was then inverted and shaken 20 times more, vertically.⁸ After decantation of the benzene extract through a fluted shark-skin filter paper into a 500-ml standard-taper Erlenmeyer flask, an additional 100 ml of benzene was added and the shaking process repeated. Both extracts were combined and worked up essentially as described previously (Gunther, 1945*b*, 1945*c*). In order to adapt this published procedure to the mass-production problems of the present experiments, however, certain short cuts and techniques were developed. These will be described later.

Manipulation for Penetration Studies. Duplicate samples of 120 average-sized mature leaves were collected, leaf areas were determined photoelectrically, and samples were weighed to the nearest gram. One sample was then stripped with benzene, as described above, to obtain the surface residue. Leaves of the duplicate sample were distributed among four pie tins lined with waxed paper, and were dried at 65° C in a forced-draft oven for 12 hours. These dried leaves were then placed in a small, clean paper bag and crushed manually by kneading. The resulting leaf fragments were transferred to an extraction thimble (60 × 180 mm) and extracted exhaustively with 350 ml of benzene until the extract had run clear for 24 hours. After filtering, this extract was analyzed for its DDT content in accordance with the procedure described in a subsequent section (see "Analytical Procedure for Estimating DDT").

⁸ The use of a mechanical stripper is advisable wherever expedient. We now use a 16-compartment drum-type stripper, which turns over at 50 r.p.m., and a stripping period of 30 minutes for standardization. The described manual stripping has proved adequate for replicated field experiments, however.

For precise work, a supplemental check procedure was devised to eliminate the possibility of carrying surface DDT inside the leaf, or vice versa, during the surface-stripping operation. In this procedure the leaves of one sample were subjected to the treatment for total DDT content. The leaves of the duplicate sample, on the other hand, were scrubbed manually on both sides with warm 10 per cent trisodium phosphate solution, rinsed thoroughly with distilled water, and then dried and extracted as described above.

With the first procedure, subtraction of the surface DDT value from the total DDT value gives the penetration value. The supplemental, second procedure serves as a check on stripping carry-in, because the value obtained when the penetration value from the leaves scrubbed with trisodium phosphate is subtracted from the total DDT value should equal the surface value obtained from the first procedure. If it does not, subsequent samples should be treated according to the second procedure, although it is too laborious and time consuming for mass production, and is to be recommended only when extreme precision is required, as in exploratory runs.

Fruit Studies

Sampling. In general, 8 pounds of most fruits constitute an adequate sample. With mature apples, avocados, grapefruit, lemons, oranges, peaches, and pears, an 8-pound sample ordinarily comprises from 10 to 30 fruits. The usual amount of DDT found on this quantity of fruit is well within the optimum range of the dehydrohalogenation method for the quantitative estimation of the DDT. It is also sufficient to minimize the various manipulation losses.

Whenever possible, the fruits were picked at shoulder height, approximately 2 pounds per tree, as widely spaced as possible. They were handled very carefully to minimize mechanical dislodgement of the existing surface deposit of the insecticide. The fruits were then placed in paper bags (nos. 16 to 20), according to fruit size, and the top of each bag was folded down and stapled shut to prevent any possibility of subsequent contamination. Samples to be used for initial surface-deposit or penetration studies were worked up immediately; otherwise, they were placed under refrigeration to await analysis. Most of the fruits studied can be held near 40° F for 2 to 3 weeks without appreciable loss of DDT.

Manipulation for Residue Analyses. Duplicate fruit samples of approximately 8 pounds each were collected and their weights determined to the nearest 10 grams. Where an analytical value of units of DDT per square centimeter was desired (rather than parts per million), it was necessary also to approximate the surface area of the sample by means of Turrell's (1946) tables. In order to do this, the major and minor axes of each fruit were determined with calipers to the nearest one-tenth millimeter. These values sufficed to locate the appropriate surface area for that fruit value in table 1 or table 2 of Turrell's book. The surface areas of fruits approximating in shape oblate or prolate spheroids, or spheres, may be determined readily according to this method.

Among the fruits studied, apples, citrus fruits, peaches, and plums simu-

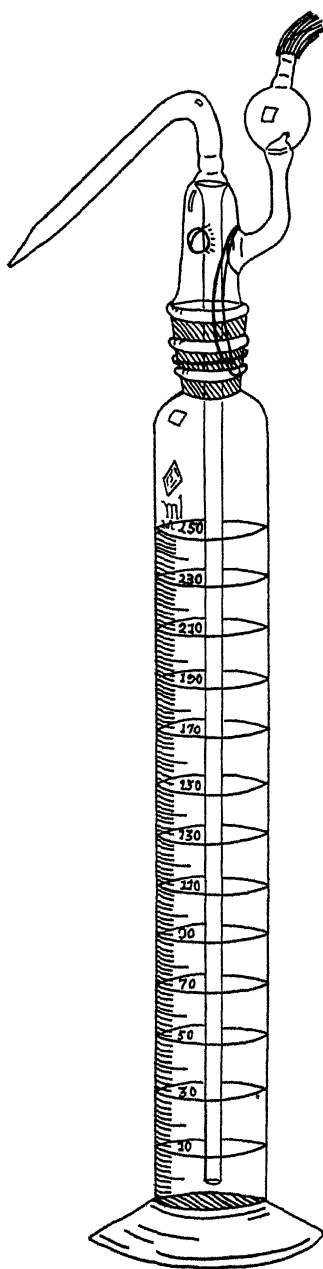


Fig. 2.—Pressurized, thumb-controlled, graduated-cylinder wash bottle used to strip deposits of DDT from fruit surfaces. (Drawing by Miss Rita Ninteman.)

late the regular shapes named above. Avocados and pears, however, present another problem. The areas of such fruits may be roughly approximated by considering each as a truncated cone placed upon a sphere, and calculating accordingly. At the present time, because of the intrinsic uncertainty underlying this geometrical approximation, an analytical value expressed in parts per million is to be considered the more cogent expression. Because of their small size, olives are usually weighed rather than measured.

For the analyses, a 500-ml standard-taper Erlenmeyer flask was fitted with a 6-inch funnel containing a 20-cm fluted shark-skin filter paper and placed on the floor of the downdraft hood. Each fruit was then pierced with an ice pick, held over the funnel, and rotated slowly. A fine stream of benzene was played over the surface of the rotating fruit by means of the pressurized, thumb-controlled, graduated-cylinder wash bottle shown in figure 2, until 15 ml of benzene had passed onto the fruit. After the entire sample had been stripped in this manner, an additional 15 ml of benzene was used to wash the filter paper and the stem of the funnel. The strip solution was worked up as described in a subsequent section.

Manipulation for Penetration Studies. Duplicate samples of approximately 8 pounds of fruit each were collected and their weights determined to the nearest 10 grams. Because the porous and thin skins of most fruits are readily permeable to benzene, and thus to a solution of DDT in benzene, it is not practical to strip fruits with benzene to remove surface deposits and then use these same fruits for penetration studies. Each fruit must therefore be scrubbed carefully with warm 10 per cent trisodium phosphate solution and rinsed thoroughly with distilled water. Obviously, the skin type determines the manner in which fruits are to be treated after scrubbing and rinsing. The further preparation of each of the various fruits studied is discussed briefly for purposes of illustration.

With all of these fruits it is well to compare yields of DDT from the steeping technique with those from exhaustive extraction, to determine the extent of DDT retention by the former more practicable method. In general, from 93 to 96 per cent of the DDT present in the dried material is transferred to the solvent with one steeping operation based upon the original excessive volume of benzene added. For example, if 500 ml of benzene is added to the dried sample, and, after steeping and gravity filtering, 200 ml of benzene is recovered, then the final DDT value obtained upon analysis is actually only about 40 per cent of the true value that would have been obtained had there been no retention of solvent in the marc.

Apples and Pears. Each fruit was cored with a standard mechanical apple corer. It was then skinned carefully with a Nee-Action Peeler^{*} whose left cutting blade had been sharpened to a razor edge and cutting slit widened to 5 mm. This type of peeling device removes a minimum of flesh when narrow strips (about 0.5 inch) of peel are removed. The strips of peel and the flesh of the fruit were then forced separately through a sausage grinder equipped with 0.18-inch perforations in the pulverizing plate. After being placed in pie tins lined with waxed paper, the ground peel and ground flesh were dried at 65° C in a forced-draft oven for 16 to 20 hours, then steeped separately with

^{*} M. and M. Mfg. Co., Chicago, Ill.

sufficient benzene to cover for 48 hours. The resulting extracts were filtered and worked up, as described in a following section, to yield values for peel content of DDT and flesh-penetrated DDT.

If the fruit is ripe, the grinding process results in a thin soup which is very difficult to dry satisfactorily, even in a vacuum oven. Ripe fruits were therefore cut into 0.125-inch slices, which were stacked carefully in the pie tins to allow circulation of air through the stack. They were then dried as above, but for 32 hours. The dried slices were trituated with benzene and then steeped for at least 48 hours.

Avocados. The thin-skinned varieties of avocado received the same treatment as apples and pears. The thick-skinned varieties were cut in half and the flesh scraped out as completely as possible with a tablespoon having one edge sharpened. Flesh and peel were then treated in the usual manner.

Citrus Varieties. The fruits were halved and each half was then reamed on a power juicer. Shreds of pulp still adhering to the inside of the hemisphere of peel were scraped out with the aid of a sharpened tablespoon. After the peel was diced, it was dried at 65° C for 16 to 20 hours in a forced-draft oven; it was then crushed and steeped with benzene in the usual manner.

Because the skin of a citrus fruit consists of two distinct portions, namely, the outer layer (flavedo) containing the oil glands and the inner spongy layer (albedo), the course of penetration of DDT into these layers was interesting to trace. For these studies, the peel was removed from lemons and oranges in longitudinal segments not more than 1 inch wide at the equatorial band. This was done with a special buttonhook-shaped peeler. Such a segment was then held flat upon a wooden block and the albedo was neatly severed from the flavedo with a Nee-Action Peeler. After a little practice, two or three strokes with the peeler usually sufficed to separate the two layers almost quantitatively. The albedo and flavedo were subsequently dried separately and extracted in the usual manner.

When semiquantitative studies of the juice and pulp were also to be made, the carefully peeled fruits were placed in a strong canvas bag and squeezed in a wine press until most of the juice had been expressed. The pulp so obtained was trituated with a little water and filtered on a Buchner funnel with the aid of a rubber dam made from a piece of inner tube. The filter cake was then dried and extracted in the usual manner. After the above filtrate had been added to the expressed juice, the resulting mixture was extracted with ether or petroleum ether (30° to 60° C). (Benzene could not be used because it formed too tight an emulsion with the juice.) The brown oil left after removal of the ether under reduced pressure was then trituated with 100 ml of benzene. After standing for several hours in the refrigerator, the benzene layer was decanted and the residue again extracted with 100 ml of benzene. The combined benzene extracts were worked up in the usual manner.

As an alternative procedure for juice and pulp analysis—when the sample was small enough—the peeled fruits were pulped with 100 ml of water in a Waring Blendor. After filtration with suction, the filter cake was repulped with another 100 ml of water and refiltered. The pulp and combined juice filtrates were then extracted as described above.

Olives. These fruits were washed thoroughly with warm 10 per cent trisodium phosphate solution to remove surface DDT, rinsed, pitted with a no. 4 cork borer, and pulped in the sausage grinder. Most of the oil was expressed from the resulting pulp in a hydraulic press at 20,000 pounds per square inch. The remainder of the oil was removed by two triturations of the press cake with petroleum ether, followed by suction filtration with the aid of a rubber dam. The filter cake was worked up in the usual manner. The expressed oil was combined with the petroleum ether filtrates, and the solvent was removed under reduced pressure. A threefold volume of benzene was added to the residual oil with vigorous agitation, and the oil was then frozen out of the mixture in the freezing compartment of the refrigerator. After decantation of the oil, the mush of frozen benzene was allowed to warm to room temperature, then was treated in the usual manner.

Peaches. After a thorough scrubbing with 10 per cent trisodium phosphate solution, the rinsed peaches were immersed in boiling water for about 1 minute, whereupon the skins were easily lifted off. The skins and flesh were then worked up separately in the usual manner. The brief immersion in boiling water apparently had little effect upon any DDT present.

Plums. No really satisfactory method for the preparation of plum samples has been devised. The stringy nature of the flesh and the high water content of the fruit have been the detrimental factors. The best and most consistent results with plums were obtained by the same treatment given to peeled citrus fruits. Vacuum drying and lyophilization were not tried, although either would probably dry the plums satisfactorily.

Analytical Procedure for Estimating DDT

The following procedure was evolved as that most satisfactory for mass DDT analyses. It is based upon the quantitative dehydrohalogenation of DDT (Gunther, 1945*b*, 1945*c*). However, since many minor modifications have been introduced to circumvent certain deficiencies encountered in the processing of many thousands of surface and penetration samples, the entire modified procedure is presented here.

Evaporation. The benzene strip or extract solutions, in 500-ml standard-taper Erlenmeyer flasks, are evaporated nearly to dryness, three at a time, on the left three units of a six-unit variable-heat extraction-apparatus hot plate. By means of an adjustable glass-tube assembly, a jet of air is caused to impinge upon the surface of the benzene solution. The heating element and the jet of air are so balanced that the evaporating benzene solution does not exceed 50° C, yet the jet of air is sufficiently gentle to prevent spattering when maintained 0.5 inch above the surface of the liquid. As the benzene evaporates, the inlet for the jet of air is lowered from time to time. This is done to maintain, approximately, the half-inch gap between it and the surface of the liquid. Across the back of the heater assembly is placed a manifold which is connected with the laboratory vacuum system. Into this is fed the emergent vapors from the evaporators. Ordinarily, on this apparatus 250 ml of sample can be reduced to a volume of about 5 ml in 10 minutes.

Digestion. To the moist residuum from the evaporation is added approximately 50 ml of 1 *N* ethanolic potassium hydroxide solution from a graduated cylinder. The flask is then placed on one of the three hot plates on the right side of the apparatus. It is fitted with a reflux condenser, and allowed to reflux gently for exactly 15 minutes, as timed by a stop clock. Three digestions may be carried on simultaneously; in the meantime fresh samples are placed on the evaporators. At the expiration of 15 minutes, the digestion flask is disconnected from its condenser, and 100 ml of distilled water is added rapidly from an automatic Machlett pipette, in order to stop the reaction. Two drops of phenolphthalein indicator solution are then added, followed by 50 ml of 2 *N* nitric acid solution from another automatic pipette. If the phenolphthalein still imparts a pink color to the solution, a few more drops of the acid solution are added. Exactly 25 ml of a saturated solution of c.p. barium nitrate is then added, with swirling, to precipitate as their barium salts any fatty acids resulting from saponification.

Propionic and butyric acids are not completely precipitated by this treatment. Where these acids appear as saponification products of the natural fats and waxes in the original strips or extracts, an alternative procedure has been developed (Beier *et al.*, 1946).

After standing at room temperature for 5 minutes or longer to allow some coagulation of the precipitated barium salt, the flask contents are filtered with gentle suction through a fluted shark-skin filter on a Fisher Filtrator into a 400-ml beaker. In order to remove water-soluble chlorides present in the paper, it is imperative that the filter paper be washed thoroughly with distilled water prior to the filtering operation. A battery of four such filters has been found most convenient for maximum efficiency.

Titration. Two drops of concentrated sulfuric acid are added to the beaker of clear or faintly turbid filtrate. The beaker is clamped into position on a warmed-up Leitz G. and D. Electro-Titrator,¹⁰ and the stirrer is started. From an automatic microburette graduated in hundredths of a milliliter, approximately 1 ml of 0.05 *N* sodium chloride is added to the sample to insure a strongly positive titrator response, even if the sample originally contained no DDT. The sensitivity knob of the Electro-Titrator is turned to 7.0, the electrode switch is turned to position 2, and the zero adjustor knob is turned until the indicating needle reads 10.0; the control knob for battery is maintained in the far counterclockwise position throughout. Then 0.05 *N* silver nitrate solution is added rapidly from another automatic microburette until the indicating needle reaches 5.9. At this point, dropwise addition of the silver nitrate is started and continued until the needle reads about 3.6, whereupon fractional drops are added to the end point 2.9.

This end point of 2.9 incorporates all corrections due to unknown factors present in all of the reagents and reactions in the entire analytical procedure. Actually, this end-point value will vary with the composition of the wet electrode, which in this instance is a calomel type containing a saturated solution of mercurous sulfate in 0.1 *N* sulfuric acid solution layered over mercury. The other electrode is silver on platinum.

From the two burette readings, the milligrams of DDT originally present

¹⁰ Gamma Instrument Company, 95 Madison Avenue, New York, N.Y.

in the sample may be calculated as follows, assuming that one mole of DDT liberates exactly one mole of chloride ion:

$$[(\text{ml AgNO}_3) (N)] - [(\text{ml NaCl}) (N)] 354.5 = \text{mg DDT}$$

If a blank value for the parent fruit or leaf sample exists, it must be subtracted from the above value to obtain the net milligrams of DDT originally in the sample.

The assumption that one mole of DDT liberates exactly one mole of chloride ion is valid only when c.p. p,p-DDT (m.p. 108° C or higher) is involved. Under the conditions specified, technical DDT of setting point around 90° C will ordinarily liberate 1.145 moles of chloride ion per mole of parent mixture. Presumably, this discrepancy is traceable to the abnormal hydrolysis of some of the o,p-isomer present to yield the chlorophenylated acetic acid derivative in addition to the desired ethylene.

EFFICIENCY OF STRIPPING TECHNIQUE FOR REMOVING SURFACE DEPOSIT OF DDT

Several samples of 120 orange leaves each from field applications of DDT were treated and stripped in the usual manner. They were then restripped. Analyses of the two separate strip solutions yielded the values shown in table 1.

TABLE 1
DDT ANALYSES OF SUCCESSIVE STRIP SOLUTIONS FROM
FIELD-TREATED SAMPLES OF ORANGE LEAVES

Sample		DDT in solution, Mg*/cm ²	
Code number	Total area, cm ²	First stripping	Second stripping
2A	5,267	1.6	0.0
3A	5,673	1.9	0.0
4B	5,301	5.8	0.0
6A	5,078	6.4	0.0
9B	5,172	8.6	0.0
8B	5,105	9.0	0.1
19B	4,516	13.3	0.2
7A	5,795	15.1	0.2

* Micrograms.

The data show that the larger the deposit of DDT, the less efficient its removal in the first stripping operation. When untreated leaves were placed in jars with added quantities (2 to 20 mg) of accurately weighed c.p. DDT (m.p. 108° C) and then stripped, recoveries of DDT by analysis varied from 98 to 101 per cent.

Mature apples, grapefruit, lemons, oranges, and pears that had been sprayed or dusted in the field with various preparations containing DDT were stripped by the wash-bottle technique and then restripped a few minutes later into separate receivers. With few exceptions, the second stripping showed no determinable DDT for these fruits (table 2).

TABLE 2
DDT ANALYSES OF SUCCESSIVE STRIP SOLUTIONS FROM FIELD-TREATED
SAMPLES OF VARIOUS FRUITS

Sample				DDT in solution			
Fruit	Code number	Total area, cm ²	Total weight, grams	First stripping		Second stripping	
				Micrograms per square centimeter	Parts per million	Micrograms per square centimeter	Parts per million
Grapefruit	2A	1,263		2.5		0.0	
	2B	1,243		2.5		0.0	
	27A	1,309		7.8		0.0	
	27B	1,271		7.7		0.1	
Lemons	LA	4,338		1.0		0.0	
	LB	7,598		1.0		0.0	
	KA	4,177		4.5		0.0	
	KB	4,130		4.6		0.0	
Valencia oranges	LA	4,780		2.4		0.0	
	LB	4,738		2.5		0.0	
	DA	5,253		9.2		0.0	
	DB	4,974		9.0		0.0	
Apples	H3A		3,888		3.7		0.0
	H3B		4,114		3.7		0.0
	C2A		3,606		11.9		0.0
	C2B		3,434		11.8		0.0
Pears	A2		7,406		6.2		0.1
	A3		7,264		6.3		0.1
	BH1		3,916		12.6		0.1
	BH3		3,887		12.9		0.2

EFFICIENCY OF TECHNIQUE FOR REMOVING PENETRATED DDT

In routine fruit analyses the efficiency of removal of penetrated DDT by means of the steeping operation was determined by exhaustively extracting one or two replicates of a steeped sample. Necessary corrections were then

TABLE 3
EFFICIENCY OF TECHNIQUE FOR REMOVING PENETRATED DDT, AS SHOWN BY ANALYSES OF
EXTRACTS CONTAINING KNOWN AMOUNTS OF C.P. DDT ADDED TO OTHERWISE
UNTREATED LEAF AND FRUIT SAMPLES

Sample			DDT		
Type	Code number	Weight, grams	Added amount, milligrams	Recovered amount	
				Milligrams	Per cent
Valencia orange leaves	1	123.5	217.3	207.4	95.4
	2	110.0	80.0	76.1	95.1
Valencia orange fruits	P5	450.0	128.6	123.2	95.8
	P3	450.0	78.2	72.8	93.1
	P1	450.0	28.6	26.9	94.0
Pears	C1	1,008.0	34.8	33.2	95.4
	C2	1,245.0	100.0	95.3	95.3

applied to subsequent steeped samples, for exhaustive extraction of large numbers of samples was not ordinarily feasible. Leaf samples, however, were always extracted exhaustively.

Table 3 shows the results of some studies to determine the efficiency of the removal of added c.p. DDT (m.p. 108° C) from otherwise untreated samples of various leaves and fruits. All samples were prepared in the usual manner, except that before the drying operation the known amount of DDT was added as a saturated acetone solution and mixed thoroughly into the sample. After the acetone had evaporated at room temperature, the usual routine treatment followed. With the leaf samples, no corrections were applied, as they were always extracted exhaustively. Fruit samples were steeped with a known volume of benzene, the volume of filtrate was measured, and the correction necessary to convert to original volume was applied.

PHOTOELECTRIC AREALIMETER

The literature of this field occasionally refers to photoelectric apparatus of one type or another, used in measuring the surface areas of irregular planar objects by means of superimposing black images of those objects on the surface of a photoelectric cell. A patient search of the literature is discouraged, however, by lack of uniformity in cataloging either these devices or the papers discussing them, in the various abstract indices. For example, one such instrument may be indexed under "area," another under "meter," another under "photoelectric," or "surface," or "surface measurement." Accordingly, it is suggested that the name "photoelectric arealimeter" be adopted for all instruments conforming to the definition suggested above. This name has not been used before in this or in any other connection, so far as can be ascertained.

Many projects in this laboratory necessitated accurate and expeditious determination of the total surface areas of thousands of leaves of citrus and of other fruits. For one reason or another, no method or instrument either mentioned in the literature or available commercially appeared adequate to this task. The planimeter, for example, was much too slow, and the method of superimposing leaves on shadowgrams of known areas¹¹ was not sufficiently accurate when slight variations in area were involved. An application of the photoelectric cell seemed the best solution to the problem.

Mention of the application of the photoelectric cell to similar problems may be found in the literature (Bulger, 1935; Kramer, 1937; Milthorpe, 1942; and others). Turrell and Waldbauer (1935) have published a bibliography on the use of photoelectric cells in plant investigations. In addition, a few photoelectric arealimeters are available commercially. All these devices were too complicated, too expensive, or insufficiently versatile.

Probably the most rigorous of our requirements was that the arealimeter must not be affected by the normal 30-volt fluctuation in our line voltage at the laboratory wall outlets. Other requirements were that the device should compensate for all green light transmitted by thin or chlorotic leaves, and that it should be completely independent of external current supply for short

¹¹ Ebeling, Walter. Unpublished data on file at the University of California Citrus Experiment Station.

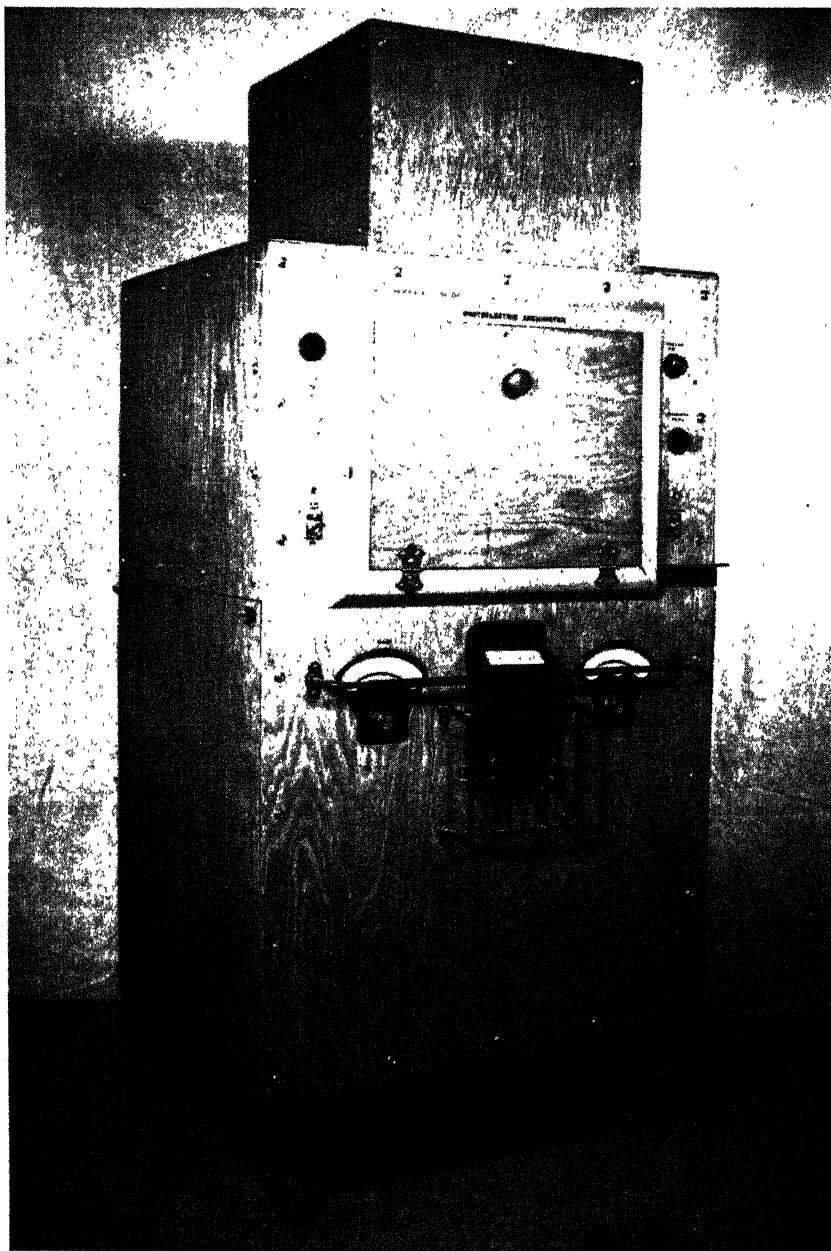


Fig. 3.—Photoelectric arealimeter.

periods of time, so that it could be transported to the field and used there when necessary. And, above all, the arealimeter had to maintain a consistently high accuracy.

All these requirements were met by the photoelectric arealimeter described herein. Free use has been made of suggestions and criticisms found in the works of the authors cited. Certain inherent "kinks" found in those units that are available commercially were carefully minimized in the present arealimeter; the nature of the improvements will become apparent as the reader considers the following descriptions.

Wiring System. A diagram of the complete wiring system of the arealimeter is shown in figure 4. This diagram is self-explanatory, except for the specifi-

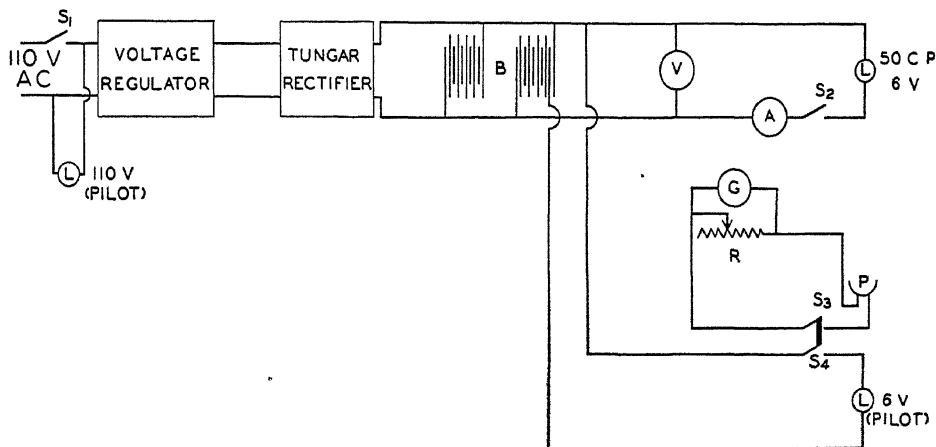


Fig. 4.—Wiring diagram of arealimeter. For description, see the text.

cations of the various instruments used. *L*, in every instance, is a light whose voltage is specified in the figure. The 50-candle-power, 6-volt light is an unfrosted automobile headlight bulb. *B* signifies two 15-plate automobile storage batteries connected in parallel. When the instrument is in continuous operation for long periods the use of four such batteries is advisable in order to decrease the frequency of supplemental recharging. *V* is a panel D.C. voltmeter, and *A* is a panel D.C. ammeter. *G* is a single-reflection, mirror-type galvanometer recalibrated from 0 to 100 in evenly spaced units, with a sensitivity of 0.06 microamperes per scale division and with a critical damping resistance of 1,000 ohms. It was necessary to rebuild the mirror suspensions and relocate the magnet of this galvanometer so that it would work satisfactorily in the vertical position required. *P* is a Weston model 594 G.B. photocell of approximately 3.5 microamperes per foot-candle, type 3S-1459M. *R* is a 500-ohm variable resistance. In addition (not shown in the diagram), a 1-ohm variable resistance is series-connected in the 50-candle-power lamp circuit in order to compensate for the slow aging of that lamp. *S*₁ and *S*₂ are single-pole, single-throw, radio-panel toggle switches, while *S*₃ and *S*₄ comprise a double-pole, single-throw switch.

Lens System. The lens system of the arealimeter is shown in figure 5. The distance between the tip of the lamp and the measuring disk of plate glass is 15 inches, and the top surface of the photocell is 20.5 inches below this plate glass. The measuring disk is 12 inches in diameter. Other pertinent dimensions

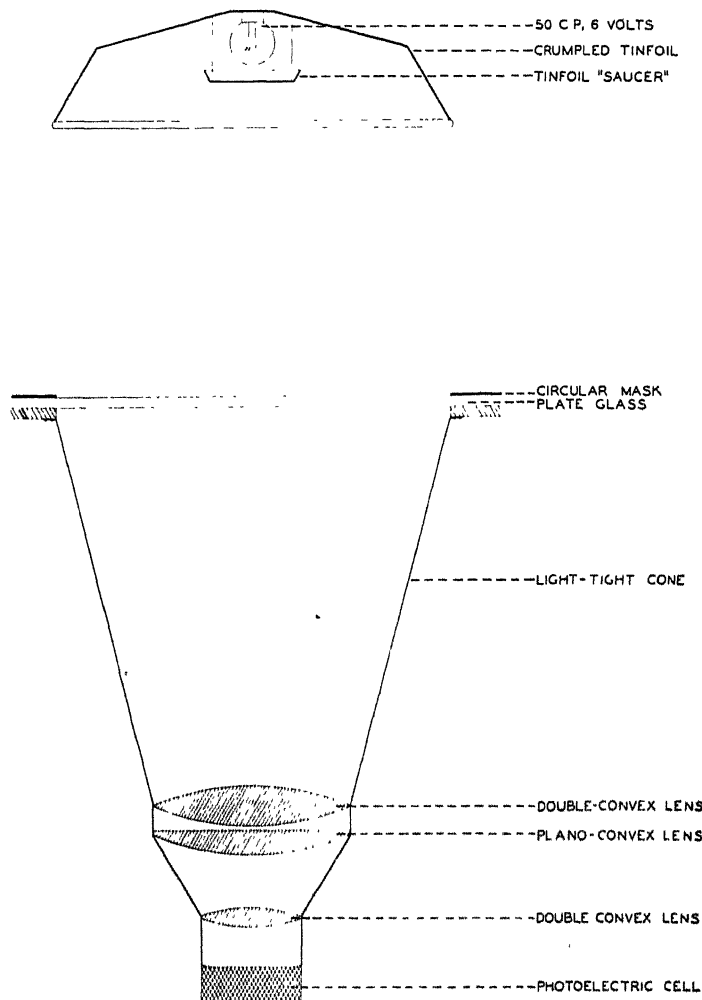


Fig. 5.—Lens system of arealimeter. For description, see the text.

are as follows: diameter of tin foil "saucer," 3 inches; distance from measuring disk to upper prism, 24.5 inches; diameter of two large lenses, 6 inches each; and diameter of small lens, 2 inches. These lenses were experimentally spaced to give the sharpest possible image of the entire 12-inch measuring disk over the entire surface of the photocell. A piece of red cellophane stretched tightly across the surface of the photocell satisfactorily excludes the green light transmitted by thin or chlorotic leaves.

Since maximum sharpness of image—and thus maximum accuracy of the instrument—depends on parallel rays of light falling on the measuring disk, the reflector indicated in figure 5 (see also fig. 6) was chosen carefully to fulfill this purpose. A large (12-inch) lens would serve equally well, but was too expensive. To eliminate undesirable highlights from the reflecting surface, and to enhance the uniformity of the light falling upon the measuring disk, the reflector is lined with crumpled tin foil. A 3-inch tin-foil saucer with upturned rim is suspended an experimentally determined distance below the bulb to exclude all direct illumination from the measuring disk. Painting the

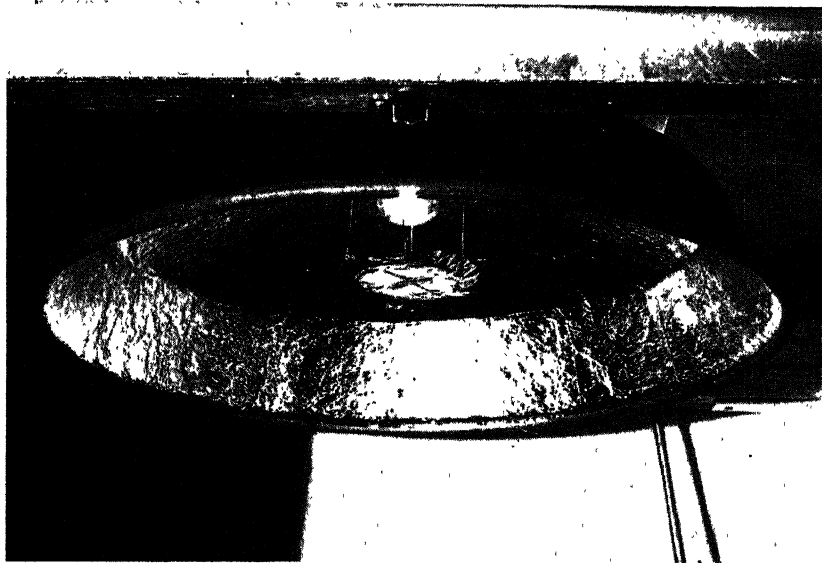


Fig. 6.—Dome-light reflector assembly of arealimeter. Attention is directed particularly to the reflecting surface of crumpled tin foil, and to the suspended "saucer," which effectively eliminates the bright spot caused by direct illumination of the measuring disk.

lower half of the bulb serves the same purposes, but the intensity of light at the measuring disk is thereby decreased materially.

Measuring Plate. The plate on which are placed all objects to be measured is shown in figure 7. The measuring disk is masked with a piece of green desk blotter beneath the plate of glass. The hinged screen, held in upright position by means of two screen-door springs (visible in the background of fig. 7), is used to hold the leaves flat on the surface of the measuring disk. A piece of rubber tubing is fitted around the rim of the reflector to prevent the screen from jarring the light assembly when released to the upright position.

Cabinet. The cabinet (fig. 3), which is built upon a welded base of quarter-inch angle iron in the form of a rectangle 20 inches long by 14 inches wide, is constructed of a well-braced framework of 2 × 2-inch boards faced with quarter-inch 3-ply veneer. A 3-inch rubber-tired steel caster is fastened to each corner of the base to make the arealimeter less difficult to move from place to place. In outside dimensions the cabinet is 51 inches high, 30.5 inches wide, and 24.5 inches deep.

Other features of construction may be discerned readily from the various photographs already discussed. The inside surface of the measuring chamber is given three coats of aluminum paint.

Occasionally it is desirable to measure leaves after they have been stripped rather than before. Most organic solvents will, under the conditions described herein, remove sufficient cuticular and other leaf waxes so that the leaves will begin to curl within a few minutes after stripping. Such leaves must be measured, therefore, while they are still wet with solvent. To alleviate the ill effects from breathing the organic solvents—and especially benzene—in such

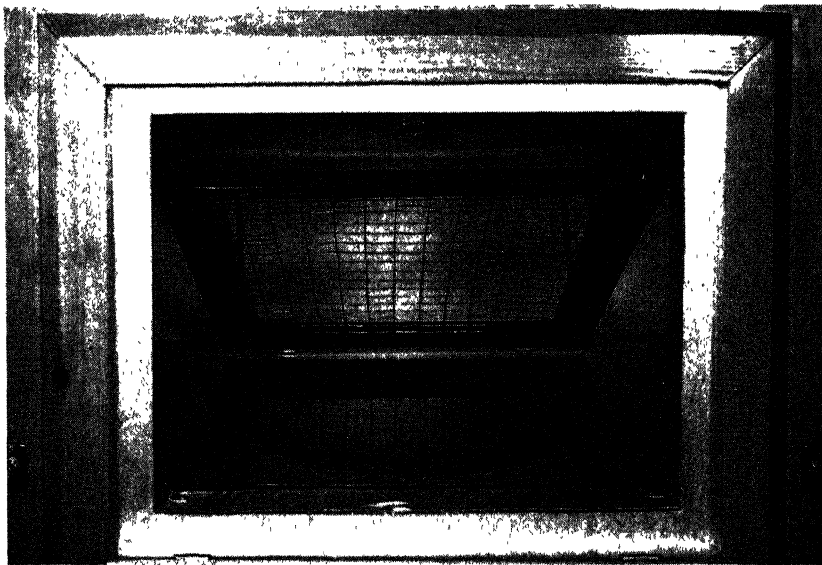


Fig. 7.—Measuring plate of arealimeter, with screen up, showing 12-inch circular measuring disk. Photographed before installation of the pilot lights.

a confined space as the measuring chamber, a 3-inch vibration-free squirrel-cage exhaust fan is mounted at the rear of the dome-light chamber. It is exhausted outdoors. The resulting gentle draft of air through the measuring chamber and away from the operator suffices as protection against the obnoxious vapors arising from the stripped leaves. Since this type of fan will starve itself in a confined space, there is no sudden onrush of air with consequent disturbance of leaves when the cabinet door is opened and closed.

Operation. To allow sufficient warm-up time, all switches (fig. 4) are turned on approximately 30 minutes before use. The galvanometer switch S_3 - S_4 is then turned off and the wire-tension control on the galvanometer housing is adjusted until the galvanometer reads 100. Switch S_3 - S_4 is turned on again, and the dome-light rheostat is adjusted until the galvanometer reads approximately zero; the final zero setting is accomplished with the 500-ohm galvanometer rheostat.

A piece of paper toweling is placed inside the light chamber to the left of the measuring disk, and the sample of leaves is dumped thereon. After the

screen has been raised, approximately 25 leaves are placed more or less haphazardly on the measuring disk. The screen is lowered and clamped, and overlapping, twisted, curled, or wrinkled leaves are straightened through the spacings in the screen. The cabinet door is then closed, and the reading of the galvanometer is noted. The screen is immediately released, the leaves are scraped to the right-hand side of the chamber onto another piece of paper toweling, and a fresh set of leaves is placed in position.

When the entire sample has thus been measured, the leaves are returned to the sample jar to be stripped, the measuring disk is wiped with one of the pieces of paper toweling, and both pieces of toweling are placed in the jar with the sample. The toweling itself contributes nothing to the subsequent DDT analyses, and it serves admirably to pick up any particles of DDT which may have been dislodged by this handling.

Addition of the galvanometer readings for the various sets within a sample, and multiplication of this sum by the constant 13.54, gives the total surface area of the sample in square centimeters. This constant was obtained as follows: Rectangular pieces of green desk blotter were cut as exact multiples of a square centimeter. These were placed in various combinations and in various places on the measuring disk, and the galvanometer reading was noted each time. A plot of galvanometer reading versus area yielded essentially a straight line within the range 0 to 730 square centimeters (full coverage of the measuring disk), whose slope was 6.77. Multiplication of a galvanometer reading by 6.77 therefore gives the single-surface area. In order to obtain the total double-surface area, this slope value is doubled. Other instruments, even though constructed as directed, would require individual calibration as above because of normal variations in photocell response.

With ordinary usage, the battery charger is left on an additional 2 hours for every hour that the arealimeter is in use. An exact balance is not possible because of the drain of the auxiliary pilot lamps, of the galvanometer lamp, and of line losses. As mentioned previously, it is not necessary for the charger to be on while the instrument is in use, if portability is desired. Under these circumstances, the batteries should be recharged for 3 hours to compensate for 1 hour of discharge. The principal reason for having the batteries in the circuit, however, is that they act as "floaters" and absorb all current fluctuations that get by the voltage regulator and the rectifier. Smooth operation of the arealimeter without these batteries is hardly possible.

SUMMARY

Because DDT applied in the field is known to penetrate certain leaves and fruits, empirical techniques for studying the magnitudes of both penetration and surface residues have been evolved and applied successfully since early 1943. The fruits considered in the present report include apples, pears, avocados, citrus varieties, olives, peaches, and plums. General techniques for the sampling and manipulation of leaves, and specific techniques for the sampling and manipulation of the various fruits, are discussed in detail.

Estimations of the DDT in or on the leaves or fruits were made with the aid of the dehydrohalogenation method of analysis, modified for efficient mass

production consistent with reasonable sensitivity. The major modification in this connection was the adoption of an electrometric titration for the chloride ion liberated from the DDT.

A detailed description is also given of the design and operation of a photoelectric arealimeter for the measurement of surface areas of irregular planar objects such as leaves.

ACKNOWLEDGMENT

The writer is indebted to those persons specifically mentioned in the text, and also to the Misses Phyllis Blair, Norma Cassina, Marjorie Elliot, Celia Lesley, Betty McFarland, Pauline Taylor, and Athalie Thomas for their aid in certain phases of the present investigation.

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THE BUD MITE AND THE ERINEUM MITE OF GRAPES¹

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IN THE COURSE of the investigation reported in this paper the writers became convinced that two and possibly three physiologically distinct strains of the grape erineum mite, *Eriophyes vitis* (Pgst.), exist on grapes in California. These are named the bud-mite strain, the erineum strain, and the leaf-curl strain in this paper. The reasons for regarding these strains as distinct, and the methods of recognizing the symptoms they produce on grapes are given in the following report.

Injury to grapevines now known to be caused by the bud-mite strain has been observed in California for at least twenty years. The minute size of the mites and the obscure nature of their attack, however, prevented the symptoms from being diagnosed definitely until 1938. In that year, H. A. Weinland, County Agent of Sonoma County, called the writers' attention to a few specimens of eriophyid mites associated with these same symptoms. The mites were sent to H. H. Keifer of the California State Department of Agriculture, who identified them as the common erineum mite, *Eriophyes vitis* (Pgst.), which had previously been associated only with irregular blisters on the upper surface of the grape leaf and felty patches of hair (erinea) on the lower surface beneath the blisters. Because the damage to the vines was so extensive and severe, and because the number of mites was so few, it seemed doubtful that the erineum mite was the causal agent.

In 1938, the writers began a series of field and laboratory observations to establish the causal relationship. They have continued these observations to the present time.

THE BUD-MITE STRAIN

Diagnostic Symptoms of Injury. During the past nine years the writers have had opportunities to study what is now known as bud-mite damage to many varieties of grapes in most of the grape-producing areas of the state. As a result, the diagnostic characters can be listed: (1) short basal internodes; (2) slight scarification of green bark of shoots; (3) flattened canes;

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Fig. 1.—Bud-mite injury on Muscat grape: Left, zigzagged stem and development of lateral shoots; right, terminal bud killed and lateral shoots developing; lower, winter spur and spring cane growth. The terminal bud has been killed, and several lateral shoots have developed.



Fig. 2.—Bud-mite symptoms of Muscat grape. Winter spur and spring cane showing first 5 nodes severely shortened.

(4) dead terminal buds on new canes; (5) witches'-broom growth of new shoots; (6) zigzagged shoots; and (7) dead overwintering buds. All seven types of symptoms are seldom found on a single vine, or even in a single vineyard. Three types of injury are usually apparent, however, and these suffice for a proper diagnosis.

The commonest symptom and surest indication of bud-mite work is the shortened basal internodes of new canes (figs. 1 to 4, incl.). The bud mites spend the winter inside the dormant spur buds which contain five to seven differentiated nodes. In some obscure manner, the mites prevent these nodes from lengthening properly when the spring growth takes place. Normally, the first six nodes should reach a total length of 12 to 18 inches. As a result of bud-mite injury, these nodes may be shortened to a total length of 1 inch. All intermediate conditions of dwarfing occur as a result of the varying intensity of bud-mite attack.

The diameter of an injured shoot, regardless of the severity of the dwarfing, remains normal. Nodes differentiating from the terminal bud after the start of spring growth always attain normal length even though growing out from severely dwarfed nodes. A slight scarification or browning of the green bark is sometimes found. The mites appear to be gregarious in habit since they are always found living close together in a colony; but this is probably caused by their habit of collecting in tight crevices which are too narrow to admit predatory mites and insects. Such colonies, by their concentrated feeding punctures, kill the epidermal layer. This scarification is usually found under the outer bud scales of dormant buds and occasionally as brown streaks on the green bark of the basal nodes of young shoots.

A symptom of less common occurrence is the development of flattened canes. Apparently, as a result of injury to the terminal bud during the winter, the cane develops with an oblong rather than a round cross section. In extreme instances, the cane may be 1 inch wide but only $\frac{1}{4}$ inch thick.

A more common type of injury is the killing of the terminal bud of the new shoot. When this occurs, the primary shoot usually attains a length of only 4 to 6 inches (fig. 1, upper right and lower). The lateral buds then push out, forming secondary shoots.

Usually one of these laterals assumes dominance and forms a nearly normal cane. Occasionally, however, five or six laterals grow equally from the shortened five or six nodes of the primary cane. This produces a bushy witches'-broom growth. In such instances, the inflorescences may form and may later produce grapes. Many times, however, the inflorescences attain a length of only 1 or 2 inches and then turn brown and fall off. Grapes formed on secondary shoots arising at the base of the primary shoots mature late. Usually, they do not attain a very high sugar content. This type of injury has been noted particularly in Thompson Seedless.

When lateral cane buds of the current year push out because the tip of the cane has lost dominance, and when these buds have been infested with mites, a peculiar and distinct diagnostic character is produced (fig. 1, upper left)—a zigzag or angular growth of the stem. Usually the stem bends sharply at the node to form about a 15-degree angle with the direction of the shoot. At the next node an equal bend is made in the opposite direction, and so on. This

produces a crooked, zigzagged shoot. Such canes lie flat since all bends lie in the same plane.

The most serious type of damage consists of killing the overwintering buds on the spurs (fig. 5). This results only from an abnormally heavy infestation of bud mites. Buds, killed in this manner during the winter, do not swell or show signs of growth the following spring. On Thompson Seedless grapes the buds most often killed are those on the basal portion of the canes.

Geographical Distribution and Host Range. The writers have seen mite damage and have authenticated the presence of bud mites by microscopic examination in Sonoma, Yolo, San Joaquin, Madera, Fresno, Tulare, and Kings counties. Hence, it is apparent that this pest is widely distributed in California, and that it is capable of producing serious damage under both cool, humid coastal conditions and hot, dry interior valley conditions. Since no survey has been conducted, it seems likely that the bud mite occurs in other grape-producing counties not listed here.

The writers have found the following varieties damaged in commercial vineyards. In each instance, the presence of bud mites was determined microscopically: Alicante Bouschet, Berger, Carignane, Emperor, Grand Noir, Black Malvoisie, Mataro, Orange Muscat, Pinot Blanc, Ribier, Sauvignon Blanc, Thompson Seedless, Tokay, White Malaga, and Zinfandel. Under laboratory conditions, the varieties Pedro Zumbon and Chardonnay were successfully infested. No evidence has come to our attention, either in the field or laboratory, to indicate that any variety is immune to bud mites.

Evidence that *Eriophyes vitis* (Pgst.) is Causal Agent. The relation between bud mites and the symptoms of injury is not easily apparent from casual observation. This relationship was carefully studied in the field, and experimental infestations were established on bench-grown vines. In the field in the spring, mites were found where symptoms of injury were detected. Conversely, mites were seldom found on vines which showed no symptoms of injury. Vineyards showing a good population of bud mites and considerable bud-mite damage were surveyed on numerous occasions for erineum on the leaves, but in the great majority of instances none was found.

Field evidence indicated that the bud mite was extensively preyed upon by several species of predatory mites, particularly *Seiulus* sp. These predators frequently reduced the bud-mite population nearly to extinction in certain vineyards. The fact that vines infested with bud mites normally developed no erineum, could be explained in three ways: (1) Sulfur dusting for mildew control may have effected a complete control of mites which wandered out onto the leaves and attempted to form erineum. (2) Such erineum-forming mites may have been killed by predators, whereas those remaining under the bud scales escaped. (3) The bud mite actually might be a distinct physiological strain in which the ability to produce erineum has been lost. To test these possibilities, mites were cultured on potted vines.

Culture on Potted Vines. Rooted vines of Pedro Zumbon, Chardonnay, and Cabernet Sauvignon varieties were set in 8-inch pots. Cuttings of Zinfandel and Mataro were rooted in sand with bottom heat, then transplanted to soil in 8-inch pots. Two cane buds on each rooted cutting were allowed to produce canes. These were tied to a stake inserted in the potted soil. Three separate



Fig. 3.—Mild injury caused by bud mite on Muscat grape. Basal nodes are somewhat shortened; inflorescences absent. Compare with figure 4.



Fig. 4.—Normal cane of Muscat grape showing length of basal nodes and the development of inflorescences. Figures 1 to 4 show canes of the same age.

tables were used to hold the potted vines. Each table was separately enclosed in a shelter 4 feet high with cellotex wire-screen roof and shade-cloth sides and ends. One table was used to rear the bud-mite strain, another table contained the erineum strain, and a third table contained check vines and a few plants infested with the leaf-curl strain (discussed later).

All potted vines were fumigated with methyl bromide before they were artificially infested with the desired strain of mites. This killed predatory mites and insects and removed all wild specimens of *Eriophyes vitis* which could contaminate the desired strain. Methyl bromide was used at 2½ pounds per 1,000 cubic feet, introduced into a 15-inch vacuum. Since this process caused considerable injury to succulent shoots, later fumigations were made using 2 pounds per 1,000 cubic feet, without vacuum, at temperatures of 65° to 68° F. Such fumigations gave a total kill of Pacific mites, *Tetranychus pacificus* McG.; grape russet mite, *Calepitrimerus vitis* (Nal.); and erineum mites and their eggs within erineae. Presumably all predatory insects and mites were also killed.

The bud mites were collected on Thompson Seedless and Malaga vines at Fresno and Madera. They were transferred to potted vines from the canes on which they were collected. This was done under a dissecting binocular microscope. Individual infested bud scales were removed and studied under the microscope for predatory mites and their eggs. If found, these were removed with a dissecting needle. The number of mites on each scale was counted or estimated. The scales, handled with fine-pointed forceps, were then wedged into the axils of leaves on the potted plants.

In all, seventeen potted vines were artificially infested in this manner, and mites were recovered from four. These four vines produced zigzagged shoots, as described under symptom number six. The first of these plants—variety Pedro Zumbon—was infested on April 6, 1945, by placing on it about 575 mites and 1,400 eggs. This plant was allowed to develop many shoots.

On May 30, 1945—54 days after infesting, mites were found in axillary buds. On August 29, 1945—145 days after infesting, this vine had two crooked dwarfed shoots. The second plant, variety Chardonnay, was infested on April 11, 1945, by placing on it 369 mites and 199 eggs. By August 29, 1945—140 days after infesting, all new shoots were dwarfed and zigzagged. The third plant, Pedro Zumbon, was infested April 30, 1945. On April 22, 1946, almost one year later, the shoots were nearly normal but showed feeding scars on their basal parts. On this date 3 live mites and a few eggs were found on this plant. The fourth plant, Zinfandel, was infested on June 21, 1945. Mites were found on this plant on July 25, 1945, and by August 29, 1945—35 days after infesting—new shoots showed typical zigzag form.

The mites living on these plants were examined by H. H. Keifer who identified them as *Eriophyes vitis* (Pgst.). No sulfur or other acaricides were put on the vines during this experiment. Predators were kept at a minimum. *No erineae developed on these plants during the year they were kept under observation.*

Consequently, it must be assumed that *Eriophyes vitis*, the well-known erineum-producing mite, has given rise to a physiologic strain which is no longer capable of producing erineum galls.

Seasonal Cycle of the Bud Mite. The bud mites pass the winter under the bud scales and as deep in the buds as they are able to penetrate. They usually are found under the second layer of scales and rarely deeper than the third layer. When vine growth starts in the spring, the mites remain for the most part under the same bud scales where they spent the winter. These scales form a whorl around the base of the new shoot.

In table 1, the distribution of mites in the spring is indicated as well as symptoms produced at that time. This table shows that up to the time the shoots are 2 or 3 inches long and have five or six leaves, the mites have not migrated out to the new buds to any extent. On the second shoot (table 1), the

TABLE 1
DISTRIBUTION OF BUD MITES ON GRAPE SHOOTS IN THE SPRING

Shoot		Bud mites		Stem zigzagged	Feeding scars		Mites up on stem
Length (inches)	Number of leaves	Number of mites	Number of eggs		Bracts	Stem	
0.25	0	200	800	—	+	+	—
1.5	4	300	500	+	+	+	+
1.5	5	75	100	—	+	—	—
1.5	4	12	27	+	+	—	—
1.5	4	20	25	—	+	—	—
1.0	3	300	150	—	+	+	—
3.0	7	200	50	—	+	—	—
0.5	1	30	10	—	+	—	—
2.0	5	9	14	—	+	—	—
0.75	2	10	10	—	—	—	—
1.0	3	5	5	—	—	—	—

+ = present; — = absent.

mites had wandered out to the axils of the leaves. Their distribution along the new shoot was: basal node, 16 mites; second node, 0 mites; third node, 2 mites; fourth node, 1 mite; and terminal bud, 0 mites. As the summer advances, the mites can be found under the scales of buds progressively farther out on the canes. Apparently the entire life cycle is completed under the bud scales since eggs, young, and adult mites have repeatedly been found there. All attempts to rear individual mites in small cages attached to leaf surfaces failed.

Identity and Probable Origin. Mites collected in the field and mites cultured on potted plants have been examined by H. H. Keifer on a number of occasions and found to be anatomically identical to the erineum mite, *Eriophyes vitis* (Pgst.). The bud mite must therefore be known by this specific name. From evidence obtained in the field and laboratory the writers believe that the bud mite is a true physiological strain which differs from the erineum strain in that: (1) it cannot produce erineum; (2) it lays eggs and breeds under the dormant bud scales; (3) it spends the entire year in the buds and axils of the leaves; (4) it may wander over the open leaf surface in migrating from bud to bud, but probably will never lay eggs on the leaves by choice; and (5) it produces the deformities described above by feeding under the bud scales, whereas the erineum strain does not produce these.



Fig. 5.—Bud-mite injury to Zinfandel. Note the length of water-sprout growth from the base of the trunk. Uninjured spur buds would have produced longer canes than these suckers.

Since the erineum strain is easily killed by dusting with sulfur, the bud-mite's habit of remaining in the buds and under the bud scales gives it a high chance of survival. It therefore seems likely that the bud-mite strain has been selected from the erineum strain by the lethal action of sulfur on the latter.

Economic Losses. From the description of diagnostic symptoms, it can be seen that the economic losses caused by bud-mite attack are: (1) loss of crop, and (2) death of the vines. Loss of crop is the predominant result of mite damage. Vines normally bearing inflorescences on two of the basal six or seven nodes are usually devoid of crop, or mature small bunches of a dozen or more berries. In vineyards where mite damage occurs, losses in tonnage of fruit often approximate 50 per cent; and the writers have seen vineyards where the crop was totally lacking. In contrast to these manifestations of severe injury, bud-mite damage often occurs on vines scattered throughout the vineyard, but is so light that it escapes the attention of the grower. In June of 1944, the following notes were made on the number of inflorescences per vine in a portion of a Ribier vineyard near Sanger:⁴

Total number of vines	92
Number of bud-mite vines	5
Per cent bud-mite vines	5.4
Number of inflorescences per normal vine	30.5
Number of inflorescences per infested vine	9.6

No more than two vines showing bud-mite symptoms were found together in this vineyard. The writers believe that the occurrence of bud-mite-damaged vines in such a scattered pattern is widespread throughout the grape-growing regions of the state. Obviously, the total acreage damaged by this pest, although certainly large, could not be accurately estimated.

The dwarfing of the basal nodes and loss of inflorescences do not adversely affect the vigor of the vines, since subsequent cane growth is usually sufficient to produce an abundant leaf canopy.

However, when the spur buds are killed during the winter (fig. 5), serious injury is done to the vines. In fact, the writers have seen practically all of the buds killed in occasional vineyards. When this occurs, serious pruning problems arise. It is necessary to establish new spurs from water-sprouts and, in some instances, new arms from the trunk. This type of injury occurring in several consecutive years seriously reduces the size of the vines. Often the main (central) growing point of damaged buds fails to grow. Shoots developing from either or both of the side growing points do not usually make such vigorous growth as those normally developing from the main growing point. Thus, the grower may have only poor canes to leave for next year's crop.

When the basal nodes of new canes are severely dwarfed, so that the total length of the first six nodes is about an inch, pruning to a suitable spur is difficult. In such instances, perhaps even the seventh and eighth nodes are left to carry the spur buds, and shoots which arise from the dwarfed nodes the following spring are removed at suckering. On Thompson Seedless grapes the overwintered buds on the basal part of the cane often fail to grow.

Control of Bud-Mite Strain. On the basis of reduction in bud-mite symptoms, the writers have seen only one case of successful control in the field. In

⁴ This data is taken from notes made in coöperation with D. F. Barnes, Bureau of Entomology and Plant Quarantine, U.S.D.A., while investigating control of the grape-bud beetle.

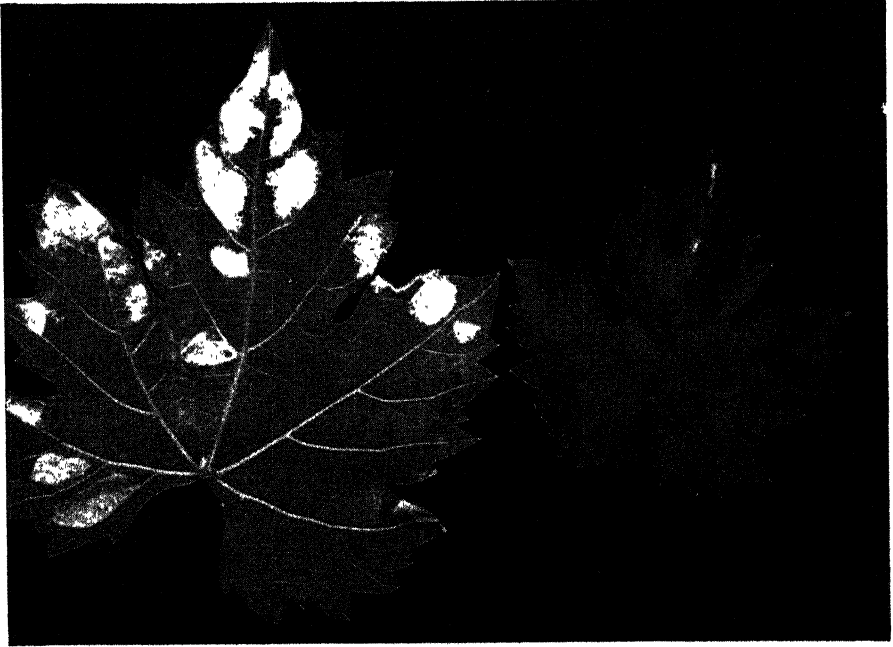


Fig. 6.—Leaves showing mature erinose galls.

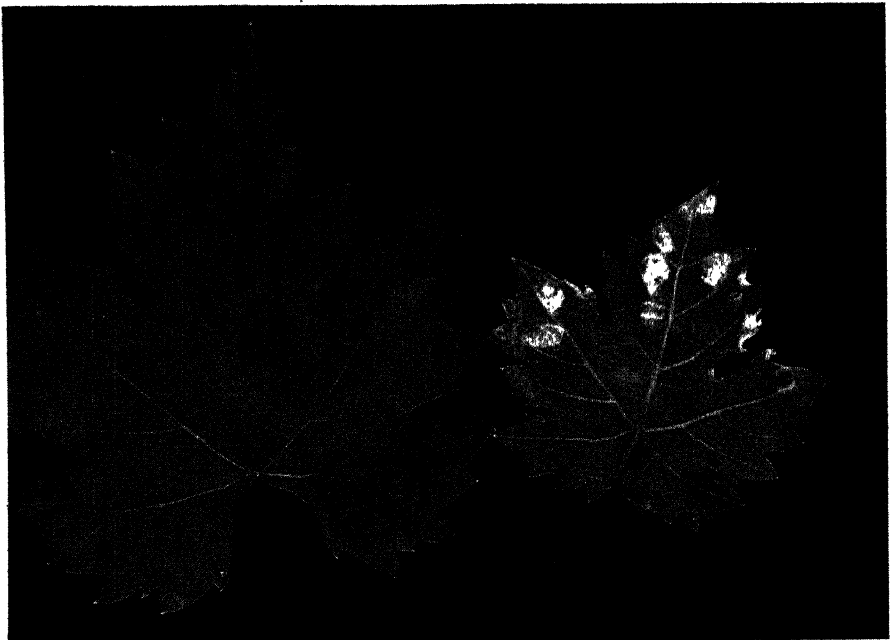


Fig. 7.—The reverse sides of the same two leaves shown in figure 6.

the fall of 1939, Mr. Weinland applied a spray composed of 3 gallons of oil emulsion and 5 gallons of lime-sulfur solution per 100 gallons of water. At the time of spraying in one section of the vineyard the vines had lost their leaves, while in another section the leaves were still on the vines. When this vineyard was examined in May, 1940, it appeared that where the spray had been applied to defoliated vines no bud-mite control resulted. In contrast, where the spray had been applied to vines still in foliage good mite control was achieved, since this section showed very little dwarfing of the shoots.

TABLE 2
CONTROL OF GRAPE BUD MITES ON EMPEROR GRAPES AT WOODLAKE
BY SPRAYS OF LIME-SULFUR PLUS WETTABLE SULFUR*

Row number (87 vines in each row)	Date of treatment	Number of vines showing bud-mite symptoms			Increase or decrease in total number of bud-mite vines	Number of mites per 25 buds
		Fall	Spring			
			Repeats	Total		
1	October 20, 1944	19	9	9	-10	28
2	October 20, 1944, and April 6, 1945	15	3	5	-10	56
3	October 20, 1944.....	10	5	16	+6	30
4	April 6, 1945.....	17	16	32	+15	12
5	April 6, 1945	14	11	19	+5	62
6	Untreated	6	4	4	-2	38
7	Untreated	4	0	2	-2	62
8	Untreated.	6	2	7	+1	..

* The writers wish to acknowledge the assistance of Norman Frazier in this test.

All attempts made by the writers to control the bud mite in the field by the application of fall or early spring sprays have been complicated by three factors: (1) Either the mites failed to appear in the spring, so that bud-mite symptoms were not apparent in treated or untreated portions of the vineyard; or (2) the symptoms appeared in some portion of the vineyard far removed from the bud-mite location of the year before; or (3) the mites appeared in such low numbers in the spring and in such unpredictable locations that interpretation of experimental results was extremely difficult.

As an example of the latter conditions, the results of an experiment to control bud mites at Woodlake are given in table 2. A portion of a vineyard of Emperor grapes was surveyed and mapped in the fall of 1944. A spray of $1\frac{1}{2}$ gallons liquid lime-sulfur, 4 pounds of dusting sulfur, and 2 ounces of $33\frac{1}{2}$ per cent dioctyl sodium sulfosuccinate per 100 gallons of water was applied October 20 to three rows of vines. Each row contained 87 vines. On April 6, 1945, when growth was very short and tender, a spray of $2\frac{1}{2}$ gallons of liquid lime-sulfur, 4 pounds dusting sulfur, and 5 ounces blood-albumin spreader per 100 gallons of water was applied to one row of vines sprayed the preceding October and to two rows not sprayed the previous October. On May 12, 1945, the vineyard was again surveyed and mapped for bud-mite symptoms. In the table of results appear only those vines showing moderate or more severe bud-mite symptoms. In the column headed "Repeats" are given the number of

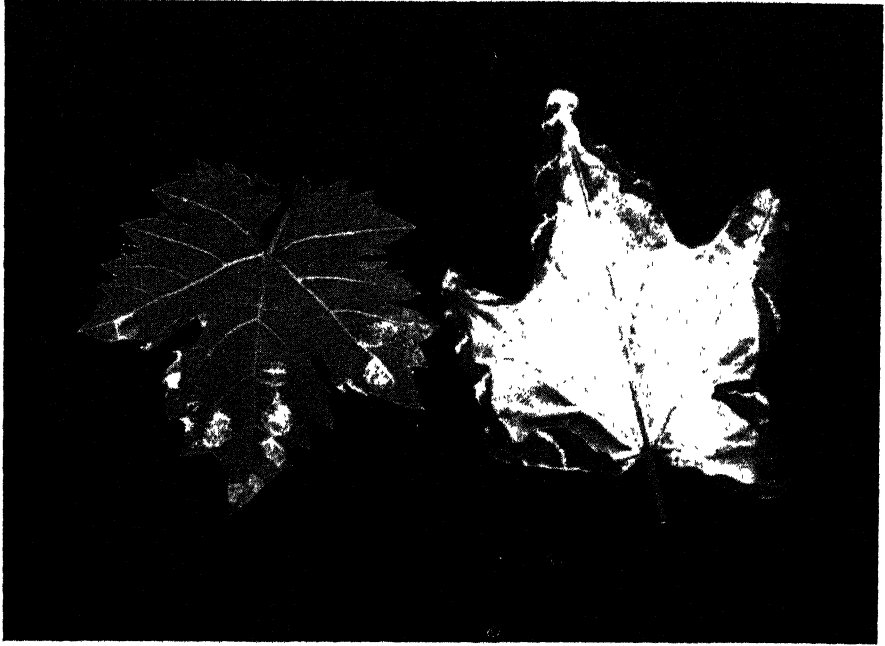


Fig. 8.—Moderate and severe erineum; erineum covers the entire lower surface of the leaf at the right.



Fig. 9.—Dorsal surfaces of leaves in figure 8. Erinose has developed along the main veins.

vines which showed at least moderate bud-mite symptoms both in the fall of 1944 and in the spring of 1945.

The table shows no clear trend in the number of vines having bud-mite symptoms. In one instance, the fall spray shows a reduction in total number of bud-mite vines; in another, an increase. This same variation is also encountered in the untreated rows. That live mites were present is indicated by the last column in the table. As the 1945 season progressed, the bud-mite symptoms were less and less noticeable. In fact, by midseason it became apparent that little, if any, damage could be attributed to bud-mite injury.

Many spray materials tested as fall sprays in other years could not be evaluated because of the erratic behavior of the bud mites. Among these were: summer oils, Selocide, ammonium polysulfide, derris extract, thialdine, and the dicyclohexylamine salt of 2,4-dinitro ortho secondary isobutyl phenol.

THE ERINEUM STRAIN

Diagnostic Symptoms of Injury. The erineum strain produces very characteristic leaf galls (fig. 7). These are nearly hemispherical in shape, with the concavity always opening on the lower leaf surface. The concavity is densely lined with abnormal curled plant hairs, whereas the upper convex surface of the gall does not differ in color or texture from the adjacent, unaffected upper leaf surface. The diameter of a mature gall is about $\frac{3}{8}$ inch. When the infestation is severe, the galls merge into a single large erinose area (figs. 8 and 9). When this occurs, the leaf may reach a diameter of about 2 inches, then die (fig. 10). Since it remains attached to the cane, the mites can leave the erineum and move to new leaves. A leaf which has 20

or 30 galls will expand to full size, and appears to carry on its normal functions. It matures and drops in the fall somewhat earlier than a noninfested leaf.

Geographical Distribution. The erineum strain is apparently distributed throughout the grape-growing areas of California, but its effects are seldom seen. Galls occasionally occur in numbers in the early spring in commercial vineyards or may be abundant throughout the season on abandoned vines or back-yard vines which are not sulfured. Wherever a normal program of sulfur dusting is followed, the erineum mite is rare to entirely absent. The writers



Fig. 10.—Severe erineum-mite damage. These leaves fail to develop further and soon die. Note erineum on the tendrils.

have found erineum in both the coastal and the central valleys; it seems equally well adapted to both types of climates.

Culture of Erineum Strain on Potted Vines. Twelve potted vines were artificially infested with the erineum strain by pinning fresh galls to the undersides of the leaves of the potted vines. All of the infestations were successful, except those on the variety Pedro Zumbon. There is some evidence to indicate that this variety may be resistant to erineum formation (as discussed under leaf-curl strain). The varieties artificially infested which developed abundant galls were Zinfandel, Cabernet Sauvignon, and Mataro. The original mites used in these tests were collected at Woodlake, California, May 12, 1945, by A. J. Winkler. When attempting to artificially establish this strain, about 50 galls were pinned to each plant. One month later, under summer-weather conditions, the test plants had developed approximately 200 galls each.

Age of Leaves Susceptible to Gall Formation. It was noted that although infested galls were pinned to older leaves, no galls developed on these leaves, but many galls appeared on younger leaves near the tips of the canes. Galls were then pinned to the three basal leaves of some plants, and a sticky band was placed between the third and fourth nodes. No galls developed on such plants.

To more accurately determine the age of the leaves susceptible to gall formation the following test was conducted. Two single-cane plants were artificially infested on each leaf. Plant A had eight leaves and plant B had eleven leaves when the mites were added. The infestation was done on June 8, 1946, and 27 days later, on July 5, 1946, the number of galls was recorded as follows:

Leaf number	Plant A	Plant B
1 (basal)	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	30	0
8	30*	0
9	8	0
10	0	15
11	—	½ galled*
12	—	⅛ galled
13	—	8
14	—	0
15 (tip)	—	1

* Indicates tipmost leaf when mites were added.

This list indicates that only the tipmost leaf and the leaf directly below it are susceptible to gall formation. In both instances, this lower leaf was about $\frac{1}{4}$ inch in diameter. Leaves $\frac{1}{2}$ inch and larger in diameter have passed the physiological stage at which the mites can stimulate gall formation. The presence of galls on leaves not present when the mites were added indicates that mites migrated from the pinned galls to the tips of the test plants for several days after infestation. The absence of galls on the youngest leaves on

July 5 indicates that migrants no longer came from the infesting galls, and, more importantly, that the mites established in new galls did not leave these to form additional galls.

Seasonal Cycle of the Erineum Strain. The adults of the erineum mite overwinter under the outer layers of bud scales of the dormant buds. As far as the writers could discover, these adults do not lay eggs and breed during the winter. As soon as the buds open in the spring, the adult mites migrate to the unfolding leaves. That two or three adults have been found in very young galls is some evidence that the mites behave gregariously and associate in small groups during the formation of the first erineum.

When first formed, the galls are bright red dorsally and white erinose ventrally. The red soon fades to yellow and then assumes the normal green of the leaf. The convex, ventral surface of the gall is covered with dense white hairs. Early in August, the ventral surface of the gall turns brown. This is caused by the death and collapse of the hairs, beginning at the dome of the gall, and proceeding slowly, over a period of several weeks, until the entire ventral surface is brown. This is not the result of feeding by the mites, since there is no evidence that they feed on the gall hairs. They apparently feed only on the lower leaf epidermis between the hairs.

When young red or yellow galls on potted plants were fumigated with methyl bromide, all of the mites and their eggs were killed. The galls then continued to develop normally and reached nearly normal size, but the hairs on the ventral concavity were sparse as compared with an inhabited gall. Evidently the course of development of the gall is determined in the first few days of feeding, but gall hairs may be provoked by the mites much later in the development of the gall. Since an undetermined number of generations is completed by the time the gall hairs die, in very populous galls the mites appear to be stacked like cordwood among the hairs.

From the middle of August to leaf drop, the adult mites migrate to the axils of the leaves and crawl in under the bud scales. This migration occurs only at night. Since the mites are very susceptible to killing by dessication, a great mortality would occur if they left the galls in the day time.

Economic Losses. Economic losses from the erineum mite are apparently very light in the state. In most instances, growers notice the characteristic symptoms on the young leaves early in the growing season. By midseason, growers show little concern for this pest.

Control of Erineum Strain. The erineum strain is easily killed by dusting with sulfur. Since all grapes are normally dusted several times a year to control mildew, the erineum mite is incidentally controlled. Where symptoms appear very early the first sulfur dust application is also made early.

POSSIBLE LEAF-CURL STRAIN

Specimens of *Eriophyes vitis* (Pgst.) were found near Modesto on August 7, 1945, on Black Corinth (Zante Currant). These mites were producing a type of injury entirely distinct from that of the bud mite or the erineum mite. Affected leaves were bowed up dorsally until nearly hemispherical. No erinose galls were present. Specimens were sent to H. H. Keifer, who identified them as *Eriophyes vitis* (Pgst.).

Mites were transferred to five potted vines, including Zinfandel, Chardonnay, and Mataro varieties. Infested leaves of Black Corinth were pinned to leaves of the test plants. In all tests the mites transferred to the potted plants successfully. They came to lie in the axils of the larger veins and at the overlap of leaf serrations. Feeding at these points no doubt had been responsible for the cupping of leaves of Black Corinth.

The artificial infestations were made on August 7, 1945. Three weeks later the mites were well established, but no leaf curl developed except with Chardonnay, on which mild symptoms appeared. A plant of Pedro Zumbon, adjacent to the infested plants, became infested and showed distinct leaf curl. The mites caused a few abnormal plant hairs to grow on spots chosen for colonizing, but no typical erinose galls appeared on the plants at any time. In the spring of 1946, these plants developed normally, showing no signs of damage by bud mite, erineum mite, or leaf-curl mite. The strain had evidently died out during the winter.

SUMMARY

Mites associated with stunted cane growth were identified as *Eriophyes vitis* (Pgst.), the common erineum mite. The symptoms commonly associated with these mites are short basal internodes, scarification of the bark of new shoots, flattened canes, dead terminal buds on new canes, witches'-broom growth of new shoots, zigzagged shoots, and dead overwintering buds. Leaf galls (erinea) are absent.

Field observations and culture on bench-grown vines indicated that these symptoms were produced by a physiological strain of the erineum mite which is herein called the bud-mite strain. This strain has lost the ability to produce, or the habit of producing, leaf erinea. It overwinters in the spur buds, and, when the new canes are about 6 inches long, migrates to the axils of the leaves and into the new buds. Field data indicate that the bud mite causes considerable loss of crop and is responsible in part for hitherto unexplained low-tonnage yields.

Parallel cultures of the erineum strain were maintained on bench-grown vines, and the habits of this strain and the symptoms it produces are described.

A single infestation of a leaf-curling strain was discovered, and some observations on this strain were made.

A SHORT METHOD OF DETERMINING LEAF AREA AND VOLUME GROWTH IN PINE TREES¹

FREDERICK S. BAKER²

SUMMARY

The determination of leaf area, leaf increment, and wood increment, and their interrelations, give a valuable insight into the basic conditions of tree growth. The methods of analysis used to date have been so expensive in time and labor that investigations of this type have been discouraged.

The chosen median twig method is described as a means of determining leaf area, leaf volume, and wood increment of twigs at a reasonable expenditure of time and a satisfactory degree of accuracy. In this method, the needle-bearing twigs of the entire crown are removed and are stratified in the following three stages:

- a. Upper crown, midcrown, and lower crown
- b. Age (the twigs being cut at annual nodes)
- c. Size

In each final stratified class an average twig is selected from a small group centering upon the median value of the class. It is shown that the twig so selected tends to be larger than average in linear dimensions, but that, since the desideratum is the twig of mean wood volume which varies with the square of the diameters, the errors come close to balancing and the chosen median twig is actually very nearly the twig of mean volume.

The leafless branches and the bole present less complex problems in sampling, and volume increment is determinable by standard methods without excessive labor.

INTRODUCTION

Many basic problems of forest growth could be approached advantageously through studies of leaf efficiency—determining the amount of solid substance produced annually per unit of photosynthetic area. Three major obstacles

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block such studies: (1) the impossibility of measuring the entire annual increment, especially that in roots, bark, and fruits; (2) the impossibility of determining the true mean photosynthetic area, which varies throughout the growing season; and (3) the enormous amount of time and labor required to strip the needles from the tree and measure or weigh them, and to analyze the growth of the innumerable twigs.

The first two obstacles are not so serious as the third. European literature (Weber, 1925,³ for example) indicates that about 80 per cent of the total weight increment of a forest tree is aboveground in the form of wood and leaves. Thus, a large proportion of total growth is readily available for measurement. The second difficulty is minor, as leaf area in evergreen trees past the juvenile stage does not vary greatly throughout the season. The third objection—that of expense and labor—seems to have rather effectively blocked study along these lines.

In spite of the laborious process, a few investigations of this type have been made. They have yielded interesting and significant evidence on a number of problems usually approached in other ways, often without particularly good success.

In Switzerland, the work of Bürger (1929, 1935, 1937, 1939, 1940, 1941, 1942) represents by far the largest contribution to the subject. It has shown that leaf efficiency of different species on the same site is surprisingly uniform, and that the common assumption that the increment of mixed stands is superior to the increment of pure stands is not substantiated. Bürger has also shown characteristic differences in the efficiency of trees of different crown classes, and has demonstrated the differences in the efficiency of tolerant and intolerant species. He has also shown that when spruce of different seed origins is planted in one place the stock of local seed origin usually gives the most efficient trees.

In this country, Hansen (1937) has analyzed, with interesting results, the increment per unit of leaf area of jack pine after thinnings. Kittredge (1944) has used the results of Bürger's, Hansen's and others' studies to find leaf area from increment and tree diameters. Other basic studies with less direct application to practice have been made by Dengler (1937), Burns and Irwin (1942), and MacDougal (1933, 1938). Very recently Möller (1945), in Denmark, has done interesting work of this type.

On the whole, the determination of the leaf efficiency of trees appears to be a valuable adjunct to other means of ecological study. It also tends to shed much light on the basic nature of forest and tree increment, particularly in its relation to age, crown classes, density of stands, and tolerance of shade. Such studies have not been made, however, largely because of the excessive amount of time required to analyze a whole tree to determine its leaf area and increment.

Although the authors cited have not generally described their methods of analysis too well, they appear usually to have undertaken the defoliation of the entire tree, after which they made a thorough analysis of growth. Bürger, however, determined increment by sampling methods. Simpler methods appear wholly feasible; one of these is described in this paper.

³ See "Literature Cited" for citations, referred to in the text by author and date.

BASIC PROBLEM

The ideal object in these studies is to determine dry weight—or volume—increment of the entire tree per unit of leaf area, since leaf area appears to be the best measure of photosynthetic capacity (Uhl, 1937). As already noted, this ideal cannot be reached as the growth of bark and roots and the production of fruits are not determinable. Both the bole wood and the current crop of leaves can be readily determined, however. These make up some 80 per cent of the total weight of the tree.

The problem of determining the volume growth of the bole is standard in forest mensuration, and the best methods are well understood. They will be merely touched upon here. The branches and twigs are miniature boles, and their volume growth is determinable in precisely the same way. They introduce a new practical element, however, for they are very numerous and small, and require an inordinate amount of time for study. Much the same is true of the determination of needle volume and area. The computation is relatively simple, but to count the needles on a tree requires much time.

The problem of determining the photosynthetic area responsible for all this bole and branch growth is, in a sense, insoluble. Some of the growth came from stored foods, laid up the season before—the product, in part, of leaves which died, fell to the ground and became mixed with leaves of earlier years. In pine trees most of the growth is doubtless due to the persistent leaves already one to three years of age, but a portion of the late summer increment must be due to the leaves that have developed during the current season. The mean photosynthetic area responsible for the season's growth is therefore indeterminable. At the same time, leaf area does not vary greatly from year to year, as the new annual suites are only a little larger than the older suites that are discarded—except in young trees. The area from mid-July to mid-September, when growth of the current year is complete and the abscission of the oldest suite has not yet begun, should give a fair index to average photosynthetic area. In young, rapidly growing trees this may not be true, as the net increase per season is very rapid. The problem then centers upon:

1. Determination of total leaf area as it appears in midsummer to late summer
2. The growth of the current year (taken during the period of dormancy, when the current season's growth is complete)
 - a. New suite of leaves (volume)
 - b. Wood volume
 - 1) In bole
 - 2) In branches and twigs

The determination of the leaf areas, the needle volume of the current year, and the growth of branchwood is the part of the work which has proved so time consuming and expensive in the past.

PRESENT STUDY

The present paper outlines a method whereby the work may be reduced to a reasonable level by means of stratification and sampling. On any large tree, there are obviously hundreds of twigs, closely similar in growth and foliage

pattern. To measure each one involves a reduplication of work to no significant purpose.

The sampling method to be described was first tried out several years ago and proved to be simple and rapid. The work, however, was done in such a manner that neither statistical analysis nor even estimation of the probable errors could be made. Accordingly, a more careful study was made involving the detailed analysis of a single, small ponderosa pine tree.

This pine tree grew at an elevation of about 2,500 feet near Placerville, California. It was 28 feet tall and 6.9 inches in diameter on the stump, was 17 years old, and was codominant in crown class. To test personal bias, independent classifications and selection of samples were made by two workers—the writer and his daughter. Although one tree is a slender basis, the evidence all indicates that the errors of the method are relatively small. Under the circumstances, this presentation is made in the hope that it may encourage the forest ecologist to explore the use of a method which will not prove too cumbersome or too expensive of time, and which should furnish results sufficiently accurate to reveal the fundamentals of forest growth in an effective manner.

TABLE 1
VARIATION IN MEASURES RELATED TO LEAF AREA AND WOOD
INCREMENT IN TEST TREE
(Based on twigs of the current year only)

	Leaf fascicles per twig	Diameter twig outside bark	Wood volume of twig
	<i>number</i>	<i>mm</i>	<i>cu cm</i>
Mean.	86.2	6.55	4.22
Maximum	405	17.5	80.9
Minimum	12	2.2	0.1
Standard deviation	65.8	2.28	10.0

Leaf Area, Leaf Volume, and Branch Increment

It has already been pointed out that the leaf and twig measurements present the practical crux of the whole problem of determining leaf efficiency, and that the crown is made up of a great number of twig units, many of which are obviously similar. Any sampling technique must then be directed toward the accurate determination of the average twig which is, specifically, the one:

1. Bearing average leaf area (since leaves vary but little in size, this means practically average leaf numbers)
2. Bearing average leaf volume for the current year
3. Having average wood increment, which means virtually:
 - a. Average volume inside bark at end of current year
 - b. Average volume inside bark at beginning of the current year

The problem may be approached either through free random sampling of the entire mass of twig material or by stratification and more restricted sampling. Since economy is a primary objective of the method, it is evident at the outset that random sampling of all the twigs of a single tree is impossible, for variance is exceedingly large, skew is pronounced, and the different items

whose mean is to be determined are not perfectly correlated. Table 1 gives data on a few typical measures to indicate the diversity of values that are encountered, while figure 2 shows the pronounced skew typical of the material. It is clear, without statistical analysis, that very heavy random sampling would be required to give a satisfactory estimate of the desired values.

Some degree of stratification is clearly needed to reduce variance, skew, and, if possible, to increase the degree of correlation between leaf area and wood increment in the sample.

Stratification of Material

The general plan of stratification has been: (1) to segregate the material from the upper, middle, and lower thirds of the tree crown; (2) to clip the leaf-bearing twigs at each annual node, to segregate the twigs of the current year, last year, the year before last, etcetera; and, in turn, (3) to sort these segregates into large, medium, and small classes. Figure 1 shows how this scheme worked out with the test tree.

First Stratification—Crown Section. The greatest single cause of wide variation in twig material and, in particular, the irregular and long-drawn-out tail of the frequency histogram on the right (fig. 2) is the small number of very large and thrifty twigs in the upper crown of the tree. The crown may, therefore, first be divided into an upper third, marked by large twigs bearing many long thrifty needles, a middle third, of less strongly developed twigs, and a lower third, composed largely of suppressed twigs with fewer and somewhat shorter needles. For convenience in handling the material, the branches cut from the tree under study should be lopped off and gathered in three piles, one from each section of the crown.

Second Stratification—Age of Twigs. Since one of the objectives of the study is to determine leaf volume produced during the current year, as well as the current length growth of twigs, the twig growth of the current season must be segregated. This is easily recognized, and can readily be clipped off with pruning shears and placed in a separate pile. The older growth may be similarly separated into internodes of last year, the year before, and so on, to promote uniformity of material.

As will be shown later, the final sampling of twigs must be done by size. Growth of the last year varies with size of the twig **only** within a group of twigs of the same age, for the larger twigs have of course grown more rapidly. With twigs of various ages a large twig may be either very thrifty or very old—if the first, current wood increment will be large; if the second, it may be very small. Accordingly, it is desirable to cut the branches at the annual nodes back at least as far as the internodes bear needles. In the particular ponderosa pine studied, this was back three years. In other trees it has been observed that sometimes four suites are retained, at least in part. Farther back, where the internodes are barren of leaves, the nodal scars become less obvious, and the increasing stem diameter renders rapid clipping with pruning shears less feasible. Such leafless branch sections can hardly be stratified effectively in this manner; they will be omitted from immediate consideration.

Each of the segregates of leaf-bearing twigs represents fairly homogeneous material, although it still varies greatly in size, with a skewed distribution of

values, as shown in the illustrative histogram for the midcrown, last year's growth class, shown in figure 3.

Third Stratification—Size. The variation can be greatly reduced, and the skew largely eliminated by classifying each age class in each crown section by size. If controlled limits are contemplated, a series of fixed gauges could be constructed by which the stems could be rapidly calipered. In this study,

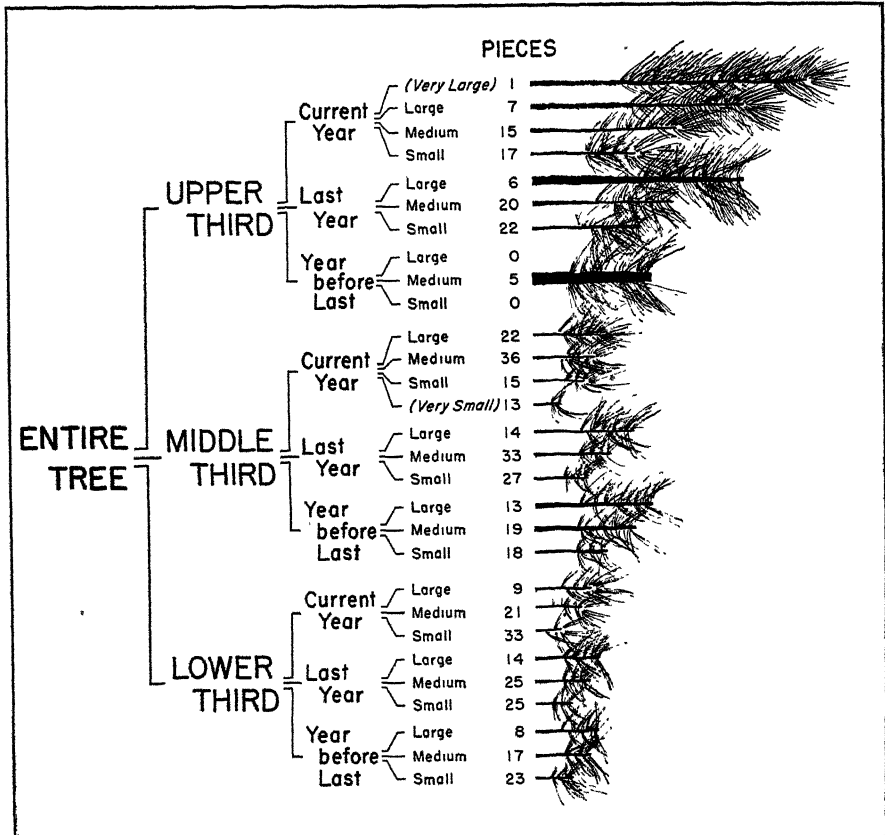


Fig. 1.—Diagram showing plan of stratification and its actual application in the test tree. Two extra categories are shown in italics. The twigs are drawn to scale as far as linear dimensions are concerned, but needles are merely suggestive.

however, economy was of great importance, and the segregation was made on the purely subjective basis of large, medium, small, with once a very large class and once a very small class.

In making the segregation, the analyst is obviously governed by many considerations—diameter, length, number of needles, and length of needles. Probably total weight would come as close to representing a concrete measure of size as anything. Weighing or any other actual measure of size slows down the analysis and offers few advantages. It is true that the size classes could be described and would have more sharply defined limits. However, since classification by diameter, for example, does not mean a clean-cut classification by

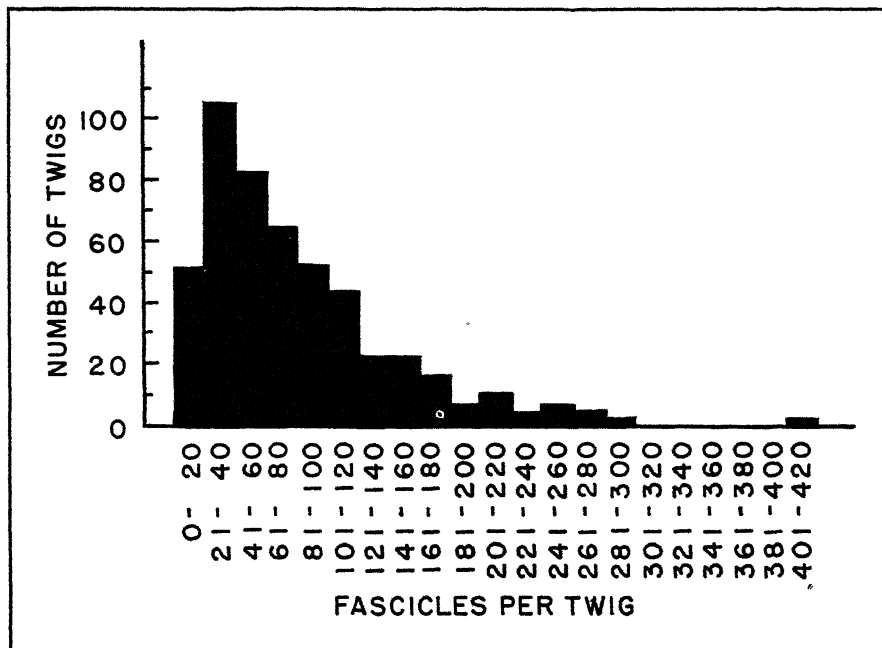


Fig. 2.—Histogram showing distribution of fascicles (leaves) per twig by 20-fascicle frequency classes.

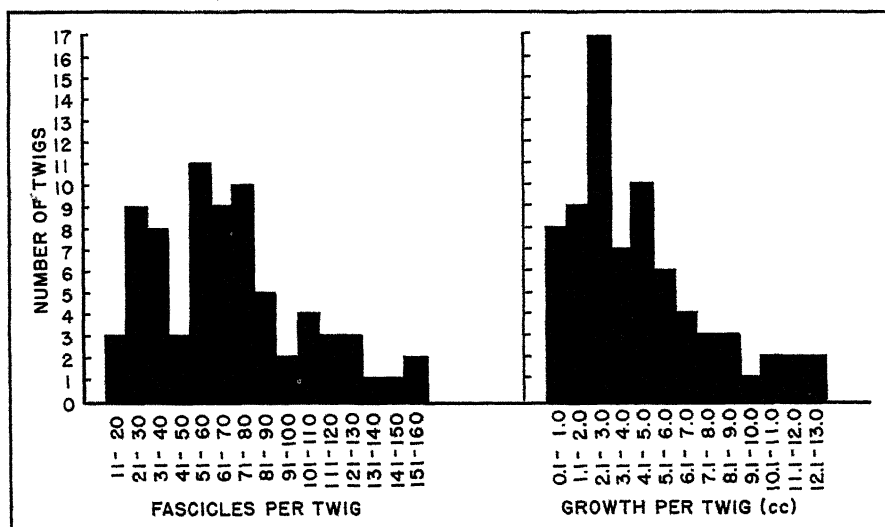


Fig. 3.—Histogram showing distribution of fascicles per twig by 10-fascicle classes and by volume per twig by 1 cc classes for the twigs of last year's growth in the midcrown section.

needle numbers, or by wood volume, or any other measure, and since none of these items is strictly correlated, little is gained thereby.

In the test tree of this study, several groups were stratified according to size by the two workers independently. The results are shown in table 2.

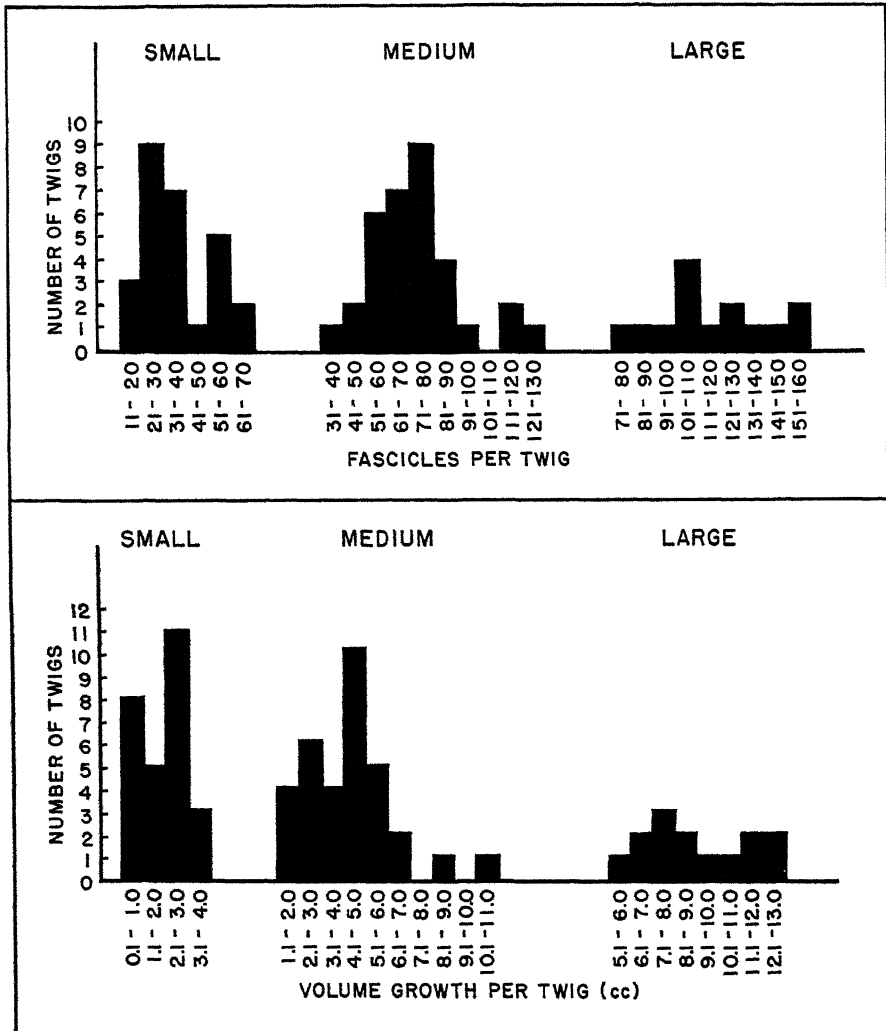


Fig. 4.—Histograms showing distribution of fascicles per twig and volume per twig resulting from the stratification of the "middle third of crown, last year's growth," into three classes based on size.

It is evident that, in the aggregate, no great differences exist between the size concepts of individuals. Even if there were considerable bias, it is doubtful whether accuracy would be reduced. The only conceivable bad effect might result from a large very-large class as against a small one. This class contains the greatest possible variance and needs to be small to promote homogeneity.

The entire leaf-bearing portion of the twigs thus becomes segregated normally into 27 groups (3 crown levels \times 3 age classes \times 3 size classes). Each group is fairly uniform as to general character and pattern of growth, has small variance, and small skew. A typical situation is shown in the class entitled, "Middle

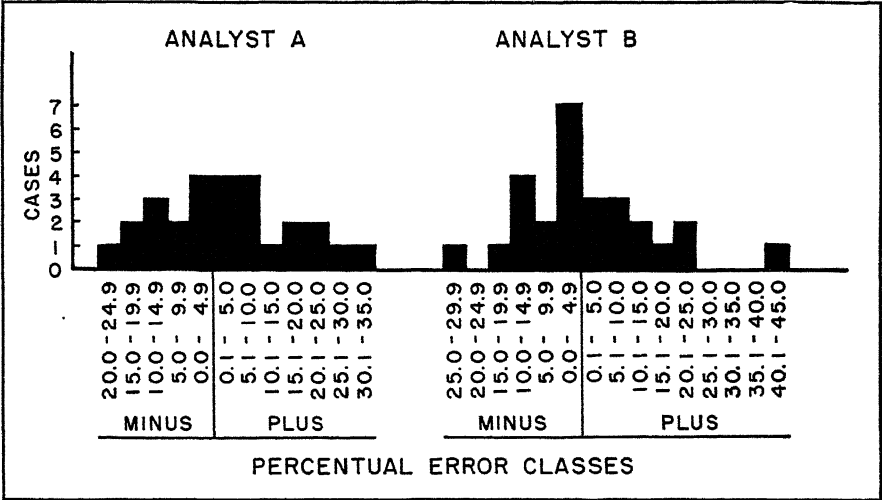


Fig. 5.—Histogram showing the distribution of percentual errors in the twig selected for average needle production by Analysts A and B.

third of crown, last year's growth" and its segregates into large, medium, and small classes (fig. 4).

Leafless Branches—Second Stratification. In the leafless branches and twigs, current wood volume increment is the single value to be secured. To

TABLE 2
NUMBERS OF TWIGS IN SIZE CLASSES AS CLASSED BY INDEPENDENT ANALYSTS A AND B

	Large		Medium		Small	
	A	B	A	B	A	B
Lower third crown, last year's	12	16	20	28	32	20
Lower third crown, this year's	9	9	18	21	37	34
Middle third crown, this year's	22	19	41	35	11	20
Lower third crown, year before last.....	9	7	18	16	21	25
Total	52	51	97	100	101	99

sample the branch wood effectively, then, it needs to be stratified in a manner that will bring variance in current wood volume increment to a low level. This increment depends upon three variables: (1) width of the last ring; (2) circumferences of the last ring; and (3) length of the twig.

The branches should first be cut up to eliminate all forked pieces. By further calculated cutting, the branches can readily be reduced to one or, perhaps, a

few groups, in each of which length variance is practically nil. The branches may then be segregated into three or, perhaps, more size groups—large, medium, and small—based on diameter at the midpoint. In such small trees analyzed to date by the author, the number of twigs in each of these categories has been very few. The average in each group has been selected by inspection of diameter and ring width, according to the general plan more elaborately applied to the leafy twig classes described later. As these leafless branches were not studied in the test tree, a more complete analysis can not be made of this class of twigs. Because the increment of the larger branches in the upper crown is considerable, the branches should be analyzed with a great deal of care.

Determination of the Average Twig

The next step is to sample each one of the segregated groups to determine, within satisfactory limits of accuracy, the twig of:

1. Mean current wood increment
2. Mean leaf area and, in twigs of current season's growth, the mean leaf volume

The approach may be either through random sampling or through a studied attempt to select the twig most nearly representative of the mean.

In either case, some standard of acceptable accuracy should be set up. Since the proportion of the wood increment of the entire tree which is laid down in these twigs is small—on the order of 1 per cent—no high degree of accuracy in wood increment determinations is called for. This is especially true of the smaller, suppressed twigs of the lower crown whose growth is so small as to be inconsequential. Under the circumstances, a standard error of the mean amounting to 10 per cent of the mean would appear amply accurate for the larger twigs in the upper crown where growth is concentrated. In the weakest twigs, 100 per cent errors would be permissible.

The determination of leaf areas and volumes requires greater accuracy, in a more uniform degree, in all parts of the crown. A standard error of no more than 10 per cent in any class would be desirable. This would result in a standard error of roughly $\frac{10}{\sqrt{27}}$ for the tree as a whole, or about 2 per cent.

Random Sampling. Accepting these levels of accuracy, the data presented in table 3 show that for a satisfactory determination of the mean needle number in each group—from which area and volume are readily computed—the random selection of about 7 twigs should be adequate. For the determination of volume increment, 15 twigs would be more nearly satisfactory—for the more important classes at least. These numbers are not large, considering the total number of twigs in each class with a tree of considerable size, yet for 27 classes they represent the analysis of several hundred twigs—no small amount of work. On this account, in spite of very real statistical advantages of random sampling, an attempt was made to select a single mean twig in each class by rather subjective means.

Selection of the Average Twig. The average twig in any class should be the twig of mean wood increment and of mean needle development. There is no assurance that the two are perfectly combined in the same twig although,

as the coefficient of correlation is 0.888,⁴ the chances are that there will be no wide discrepancy.⁵

The identification of the average twig is not simple. The current wood increment—the volume of the outermost ring—varies with the diameter of the twig, the thickness of the ring, and the length of the twig. It is impossible for the mind to size up subjectively the volume of such complex and “invisible”

TABLE 3
NUMBER OF TWIGS, SAMPLED AT RANDOM, REQUIRED TO OBTAIN STANDARD ERROR OF
10 PER CENT OF THE MEAN

	Needles on twigs of			Volume increment twigs of		
	Current year	Last year	Year before last	Current year	Last year	Year before last
Upper third crown						
Large and very large	7 1	2 6	.	18 2	7 7	.
Medium	2 1	5 9	22 7	4 3	16 1	36.5
Small	27 6	20 1		27 2	23 0	
Middle third crown						
Large	3 3	5 0	4 7	10 5	7.3	11 7
Medium	4.6	7 3	8 5	6 3	19 0	18.2
Small and very small	15 4	17 2	25 6	8 7	29 2	31.2
Lower third crown						
Large	7 0	5 8	7 7	11 0	14.7	10 7
Medium	4.1	6 8	12 2	2.8	8.9	17 7
Small	14 0	20 2	27.6	9 5	22.2	15 6

solids. The leaf area problem is simpler. Needles vary little in length and diameter within a single twig size class. Needle number is the chief variable, and that can be judged fairly well.

The twig of average wood increment must therefore be chosen indirectly. The chief sensible item correlated with volume growth of wood is twig diameter (within a given age class), for large twigs of the same age will naturally tend to have greater annual increment than small twigs. This is not rigidly true, for the outermost ring may be abnormally large or small, and may be offset by abnormally small or large rings of earlier years. In general, however, twig growth is uniform and, accordingly, large twigs consistently mean large increment, as shown by the following correlation (coefficient of 0.242 is significant at the 1 per cent level) :

Diameter outside bark

and diameter inside bark..... 0.9517
and twig length 0.9267
and diameter inside bark a year ago..... 0.9181
and wood increment (last ring) 0.8875

The twigs in each group were therefore classed roughly by size, using again the rough general impression that is analogous to total weight.

In performing this classification, the twigs were spread out in a line, with

⁴ This was determined on the basis of top third, current season's growth, and midthird, last year's growth only. The value appears to be characteristic, however.

⁵ With 113 the degrees of freedom in this case, correlation is significant at the 1 per cent level when the coefficient is 0.242.

the smallest on the left and the largest on the right. Then, by counting off from one end, the median 5 twigs were taken out of the group and were carefully considered from a standpoint of characteristic foliage (length and number of needles), average bark (giving normal diameter inside bark), and ring width. Out of the 5, the best single one was chosen.

With this purely subjective selection it becomes difficult to discuss the value of the chosen median twig, as representative of the average, in terms of statistical theory. There are too many complex interrelations. The best evidence of the feasibility of the method must come from the evidence of the test tree.

Estimation of Number of Leaves

The determination of the number of leaves on the test tree by the chosen median twig method used by the two analysts independently gave results that were slightly high in both cases. A's aggregate estimate was $1.9 \pm .1$ per cent high and B's was $1.3 \pm .1$ per cent. This aggregate error is fairly satisfactory, but a more careful analysis is needed to determine means of improving accuracy and to avoid gross errors. The details of the analysis are shown in table 4, an examination of which shows large percentual errors in line 5 (A), 11 (A and B), 12 (B), 17 (A and B), 20 (B), 27 (A).

A careful study of the field records disclose several causes of these large errors:

1. In small groups of twigs (line 5, 6 twigs, for example) no single twig closely resembles the mean.
2. It is difficult to find specific twigs that are median both in foliage (fascicle numbers) and in wood growth. This is the cause of most of the errors, as may be seen in table 5, where the twigs in groups with large leaf errors frequently have relatively small wood increment errors.
3. The preponderance of plus errors appears to be natural and unavoidable. If the average number of needles per twig is 30, a twig with only 20 needles looks strikingly thin, but one with 40 does not look correspondingly overproductive of leaves.

Estimation of Wood Increment in the Test Tree

As already described, the selection of the twig of average increment for the current year was made in each of the 27 stratification groups by choosing the twig of average size and ring width. Diameter of outside bark and twig length are the major sensible dimensions upon which a choice of this kind is naturally founded. At the same time, the five semifinal median selections were also examined to determine whether bark and rings appeared to be of normal development. Small abnormalities are hardly discernible, being almost microscopic in twigs whose outside diameters are no more than 2 to 3 millimeters. Essentially then, the choice was made on the assumption that the twig of average diameter and length has average volume growth. This is, of course, flatly untrue.

In twigs of the current year's growth, which may be considered frustums of cones, volume may be expressed most readily by the formula known to foresters as "Huber's formula":

$$V = \pi R^2 L$$

where V is the volume, R the radius of the twig inside bark at a point halfway along the twig length, L . It is evident that in any group of twigs of varying size the twig of mean volume will have greater than mean diameter. This

TABLE 4
ERRORS IN DETERMINATION OF LEAF NUMBERS FROM CHOSEN MEDIAN TWIGS

Stratification class	Line number	Twigs	Number of fascicles	Error of chosen twig			
				Fascicle number		Per cent	
				A	B	A	B
Entire tree.	35,726	+687	+462	+ 1.9	+ 1.3
Top third.						± .1	± .1
Current year							
Very large.	1	1	405	0	0	0	0
Large.	2	7	1,590	- 30	- 30	- 1.9	- 1.9
Medium	3	15	2,550	+ 76	+225	+ 2.9	+ 8.8
Small	4	17	1,751	-391	-306	-22.4	-17.5
Last year							
Large.	5	6	1,308	+342	- 12	+26.0	- 1.9
Medium	6	20	2,540	-280	-280	-11.0	-11.0
Small.	7	22	2,270	-180	- 26	- 7.9	- 1.3
Year before last							
Medium.	8	5	1,130	+ 45	+ 45	+ 4.0	+ 4.0
Middle third							
Current year							
Large	9	22	2,609	+251	+ 75	+ 9.6	+ 2.9
Medium	10	36	3,082	+158	- 58	+ 5.1	- 2.3
Small.	11	15	803	+202	+202	+23.9	+23.9
Very small.	12	13	381	+ 9	-108	+ 3.5	-27.5
Last year							
Large	13	14	1,638	-210	-210	-12.8	-12.8
Medium.	14	33	2,376	+264	-165	+11.1	- 7.1
Small	15	27	972	- 27	+108	- 2.8	+11.1
Year before last							
Large	16	13	1,352	- 52	- 52	- 4.0	- 4.0
Medium	17	19	1,387	+323	+323	+23.0	+23.0
Small.	18	18	558	+54	+144	+ 9.6	+12.5
Lower third							
Current year							
Large.	19	9	717	+ 39	+ 39	+ 5.2	+ 5.2
Medium.	20	21	1,098	-153	+519	-13.5	+48.0
Small	21	33	981	+ 9	-123	+ 9	-12.3
Last year							
Large.	22	14	868	-140	- 28	-16.1	- 3.1
Medium.	23	25	964	+186	+186	+15.2	+15.2
Small.	24	25	641	- 91	- 66	-15.4	-11.5
Year before last							
Large	25	8	574	- 30	- 30	- 5.5	- 5.5
Medium	26	17	691	+159	+ 74	+19.0	+ 7.2
Small.	27	23	490	+154	+ 16	+33.3	+ 3.7

occurs because the squaring of the radius disproportionately enlarges the volume of the bigger twigs and correspondingly penalizes the smaller.

For example, in the class shown in line 7, table 5, the mean diameter outside bark is 9.51 mm, inside bark is 6.33 mm, and inside the last ring is 3.93 mm. Twig length is 245 mm. The volume outside bark of the twig of average diameter and length is 17.44 cc, but the average volume of the twigs in

the class is 18.85 cc, a difference of 1.41 cc' (8.1 per cent). Similar differences exist in the volumes inside bark, at the present time and a year ago, so that the increment of the twig of average linear dimensions is 4.68 cc, although the mean volume increment is 5.27 cc. Thus, the twig of mean linear dimensions will have less than mean volume.

TABLE 5
ERRORS OF WOOD INCREMENT DETERMINATION

Stratification class	Line number	Pieces	Volume cc	Error			
				A cc	B cc	A per cent	B per cent
Entire tree.	3,084	+ 59	+141	+1.91	+4 56
Top third							
Current year							
Very large.	1	1	81	0	0	0	0
Large.	2	7	280	+ 6	+ 6	+ 2.0	+ 2.0
Medium	3	15	170	+ 15	+ 3	+ 8 8	+ 1.8
Small.	4	17	88	- 10	- 20	-11.3	-22.7
Last year							
Large	5	6	327	+ 3	+ 69	+ 0 9	+21.1
Medium	6	20	244	+ 56	+ 56	+23 0	+23 0
Small	7	22	114	0	+ 3	0	+ 2 6
Year before last							
Medium	8	5	180	- 5	- 5	- 2.8	- 2.8
Middle third							
Current year							
Large.	9	22	674	+ 8	+ 8	+ 1.2	+ 1 2
Medium.	10	36	89	- 13	+ 26	-14.6	+29 2
Small	11	15	15	- 3	- 3	-20 0	-20.0
Very small.	12	13	2	0	0	0	0
Last year							
Large	13	14	119	- 21	- 21	-17.6	-17 6
Medium	14	33	142	- 3	- 20	- 2.1	-14.1
Small	15	27	43	+ 14	+ 19	+32.5	+44 2
Year before last							
Large	16	13	148	+ 15	+ 15	+10.1	+10.1
Medium	17	19	129	- 2	- 2	- 1.6	- 1.6
Small	18	18	38	- 24	+ 7	-63 2	+18.4
Lower third							
Current year							
Large	19	9	20	- 8	- 8	-40.0	-40 0
Medium.	20	21	18	+ 12	+ 5	+66.7	+27.7
Small.	21	33	10	- 7	- 3	-70.0	-30.0
Last year							
Large	22	14	35	- 21	+ 7	-60.0	+20.0
Medium.	23	25	25	0	0	0	0
Small.	24	25	13	- 5	+ 3	-38.4	+23.1
Year before last							
Large	25	8	21	+ 5	+ 5	+23.8	+23.8
Medium.	26	17	34	+ 22	- 8	+64.7	-23.5
Small	27	23	35	- 2	- 10	- 5.7	-28.6

It becomes impossible to define the twig of mean volume growth in any practically satisfactory way, because mean volume growth may occur in a large twig with a small current ring or a small twig with a wide current ring. It is certain, however, that a twig of average diameter, average bark thickness, and average width of the current ring will have less than average volume.

In this confusing situation, we may cut straight to the crux of the problem, and observe first of all how well the chosen twigs in the test tree represented the twig of mean volume increment in each of the 27 stratification classes.

As shown in table 5, the sampling was successful on the whole since, for the entire tree, A's error was about 2 per cent and B's, roughly $4\frac{1}{2}$ per cent. A detailed examination of the table shows large and irregular percentual errors in the lower third of the tree and in the small categories in the middle third. These errors are not serious. They appear to be generally fortuitous and are largely compensating, and involve twigs which carry less than 1 per cent of the entire growth of the tree.

By far the most important errors are those made by both A and B on line 6 and B's large error on line 5. An examination of the original data shows that the first of these was occasioned by the selection of a mean sample twig which had a current ring 2.0 mm wide against an average of 1.5 mm. In the second instance, the current ring was about 0.3 mm wider than the average of the group, and twig length was also somewhat excessive. It is hardly to be supposed that occasional errors of this magnitude can be avoided.

It will also be noted that A had 5 errors in excess of 60 per cent. B's highest was 44 per cent. It happened that these large errors were chiefly compensating, but the effect is to give A a mean error of 21.8 ± 6.1 per cent and B one of 16.6 ± 3.9 per cent.⁹ This suggests a somewhat higher order of judgment by B than by A. An examination of the data, however, indicates that the more likely reason is that A gave more weight to needle numbers in picking the median sample twig while B considered twig volume more carefully.

Examining the 25 cases in which errors of 10 per cent or greater exist, the most important reasons appear to be as follows:

	Per cent
Poor choice, too large or too small outside bark	32
Bark exceptionally thick (volume of wood low)	4
Current ring broader than average	20
Current ring narrower than average	25
Twig much longer than average	4
Twig much shorter than average	8
Poor stratification (excessive variance)	4
Small class, no actual average twig available	4

It is evident from this analysis that no one thing is strikingly at fault, but, in order to gain a more complete picture of the situation, a somewhat more detailed analysis is required.

Diameters of Selected Median Twigs. In order to study the characteristic errors in the diameter of the selected median twig, it is necessary to use the true mean diameter of the class from which the twig is selected as a fixed norm of comparison. But again it must be emphasized that this mean diameter is the diameter of a twig of less than mean volume. The discrepancy between the diameter of a twig of mean volume and that of mean diameter depends upon the degree of variance in the class. On the whole, however, in the material from

⁹ These figures are the arithmetical means of the values in the last two columns disregarding algebraic sign, with corresponding standard deviation of the mean.

the test tree, the twig of average volume has about 0.2 mm larger diameter than the twig of average diameter based on same twig length in both cases (table 6). The greatest differences between the two appear in the classes where

TABLE 6
DIFFERENCES BETWEEN THE TWIG OF MEAN VOLUME AND THE TWIG OF
MEAN DIAMETER WITH RESPECT TO DIAMETER INSIDE BARK (D.I.B.)
AND DIAMETER INSIDE BARK A YEAR AGO (D.I.B.-1)*
(Computed on basis that both have same length)

Stratification class	D.I.B. mm	D.I.B.-1 mm
Upper third		
Current year		
Very large.....	0	.
Large.....	.3	..
Medium.....	0	..
Small.....	.2	..
Last year		
Large.....	.2	.2
Medium.....	.4	.3
Small.....	.3	.2
Year before last		
Medium.....	.1	.2
Middle third		
Current year		
Large.....	0	..
Medium.....	.4	..
Small.....	.3	..
Very small.....	.1	..
Last year		
Large.....	.1	.3
Medium.....	.2	.2
Small.....	.2	.4
Year before last		
Large.....	.4	.3
Medium.....	.3	.2
Small.....	.3	.2
Lower third		
Current year		
Large.....	.5	..
Medium.....	.3	..
Small.....	.6	..
Last year		
Large.....	.4	.2
Medium.....	.3	.5
Small.....	.2	.1
Year before last		
Large.....	.1	.2
Medium.....	0	0
Small.....	.1	0
Average.....	.22	.22

* Twig of mean volume is always larger.

the twigs are very small and where variance—on a percentual basis—is correspondingly large.

Table 7 shows the accuracy with which the twig of mean diameter is selected by the analysts. There is a consistent tendency to pick twigs which are a little

larger than the average. Since the actual visible evidence of diameter is that outside of bark, it is perhaps the most important diameter measure to inspect for bias and gross error.

TABLE 7

ERRORS IN DIAMETER BETWEEN THE CHOSEN MEAN TWIG AND THE TWIG OF AVERAGE
DIAMETER OUTSIDE BARK (D.O.B.), INSIDE BARK (D.I.B.) AND
INSIDE BARK A YEAR AGO (D.I.B.-1)

Stratification class	Line number	Diameter chosen twig minus average diameter of class (mm)					
		D.O.B.		D.I.B.		D.I.B.-1	
		A	B	A	B	A	B
Upper third							
Current year							
Very large..	1	0	0	0	0
Large	2	+1 0	+1 0	+ .3	+ .3
Medium	3	+ .8	-1 5	+ .2	+ .3
Small	4	+ .3	-1 8	- .2	- .5
Last year							
Large	5	+ .3	+ 3	+ 5	+ .7	+1 2	+ 2
Medium	6	+1.9	+1 9	+ 7	+ .7	- .3	- 3
Small	7	0	+ .5	- .3	+1.1	- .6	+1.4
Year before last							
Medium	8	- .6	- 6	- .3	- .3	- .5	- 5
Middle third							
Current							
Large	9	+ .1	+ 3	+ .1	+ 1
Medium	10	+ .3	+ .2	+1.0	0
Small	11	+ .1	+ .1	+ .2	+ .2
Very small	12	+ .2	+ .1	+ .1	0
Last year							
Large	13	- .2	- .2	- .3	- .3	-1.0	-1.0
Medium	14	0	+ .3	+ 1	+ .1	+ .4	+ .2
Small	15	+1.4	+ .7	+1.0	+ .4	+ .6	- .1
Year before last							
Large	16	+ .7	+ .7	+1.2	+1.2	+ .8	+ .8
Medium	17	-1 5	-1.5	- .3	- .3	- .9	- .9
Small	18	-1.5	- .4	-1.2	+ .1	-1.1	+ .1
Lower third							
Current							
Large	19	- .1	- .1	- .6	- .6
Medium	20	+ .8	+ .2	+ .4	+ .5
Small	21	+ .2	+ .1	0	- .2
Last year							
Large	22	+ .2	+ .4	- .5	+ .5	+ .4	- .1
Medium	23	+ 3	+ .3	+ .3	+ .3	+ .8	+ .8
Small	24	- .2	+ .5	+ .1	+ .4	+ .6	- .4
Year before last							
Large	25	+1.0	+1.0	+1 2	+1.2	+1.0	+1.0
Medium	26	+ .3	- .4	+ 9	+ .2	+ .6	+ .6
Small	27	+ .2	- .2	0	- .5	- .1	- .4
Average	+ .22	+ .07	+ .17	+ .20	+ .07	+ .09

In the 27 cases, A shows 18 plus errors, 3 zero errors, and 6 minus errors. B's errors are similarly divided—17 are plus, 1 is zero, and 9 are minus. The largest errors are in line number 6, where large volume errors also occurred (table 4), the error being nearly 2 mm (in a twig of 12 mm outside diameter).

On the whole, it cannot be said that errors are either large or erratic. They do reflect, however, a definite tendency to select specimens somewhat larger than average.

TABLE 8
ERRORS IN LENGTH OF SELECTED TWIGS COMPARED WITH THE MEAN LENGTH
OF TWIG IN EACH CLASS

Stratification class	Line number	Mean	Error in length of twigs			
			Number		Per cent	
			A	B	A	B
Whole tree						
Top third						
Current year						
Very large.	1	610	0	0	0	0
Large...	2	475	+ 5	+ 5	+ 1.1	+ 1.1
Medium	3	327	+13	-20	+ 4.0	- 6.1
Small.	4	238	+17	+12	+ 7.1	+ 5.0
Last year						
Large...	5	470	+30	+20	+ 6.4	+ 4.3
Medium	6	317	-11	-11	- 3.5	- 3.5
Small..	7	245	+10	+ 5	+ 4.1	+ 2.0
Year before last						
Medium .	8	264	-14	-14	- 5.3	- 5.3
Middle third						
Current year						
Large...	9	148	+ 2	- 3	+ 1.3	- 2.0
Medium.	10	140	0	0	0	0
Small..	11	120	-17	-17	-14.2	-14.2
Very small.	12	65	+ 5	+20	+ 7.7	+30.8
Last year						
Large...	13	229	-59	-59	-25.8	-25.8
Medium .	14	178	+22	+22	+12.3	+12.3
Small.....	15	124	-11	+15	- 8.9	+ 8.3
Year before last						
Large...	16	263	-23	-23	- 5.7	- 8.7
Medium.....	17	230	-35	-35	-15.2	-15.2
Small..	18	162	-58	+36	-35.8	+22.2
Lower third						
Current year						
Large...	19	122	- 7	- 7	- 5.7	- 5.7
Medium .	20	106	+29	+ 4	+27.3	+ 3.8
Small..	21	60	-10	+25	-16.7	+41.7
Last year						
Large...	22	142	-17	+ 8	-12.0	+ 5.6
Medium...	23	127	+27	+37	+29.0	+29.0
Small.....	24	81	-11	+ 9	-13.6	+11.1
Year before last						
Large...	25	141	- 6	+ 6	- 4.3	- 4.3
Medium..	26	123	+42	+12	+34.2	+ 9.8
Small.....	27	85	+18	0	+21.2	0
Average..	-1.8	+1.3

Diameters inside bark show a roughly similar trend. Both A and B have an average error of plus 0.2 mm, but again the errors are neither very large nor erratic. Diameters inside bark of the test twigs a year ago average about 0.08 mm too large.

We have already noted that the twig of mean volume in any segregated class has a diameter greater than the average of the class. A comparison of the data in tables 6 and 7 shows whether the selection of an inordinately large twig corrects or overcorrects this. As far as diameters inside bark are concerned, the average error indicates that the chosen median twig method almost precisely corrects the error. In the case of diameters a year ago, the error of only about 0.08 mm, occasioned by picking a twig somewhat too large, is insufficient to correct the error of 0.22 mm in the opposite direction. The last ring is therefore computed a little too wide and volume is a little too high. These errors are small, however, and rest upon a dimension (diameter a year ago) which can be given little subjective weight in selection. The errors therefore seem of relatively minor statistical significance.

The distribution and nature of errors in the length of the median selected twig are shown in table 8. These errors are rather large percentually in the smaller twigs. For example, A's error of 42 mm in a class whose mean length is only 123 mm (line 26), errors of both A and B on line 23, A's error on line 18, are all considerable on this basis. As pointed out before, the volume growth of these small weak twigs comprises a very minor part of the increment of the entire tree, and large errors are permissible. On the other hand, the errors in the large twigs are generally small—under 10 per cent.

It is possible that some of this error is deliberate—that is, the analyst selects a twig somewhat longer than average to compensate for an apparently small diameter. A check on this shows that in 34 cases (A and B) in which the chosen twig has a plus diameter error, the length errors are also plus in 21 cases. In the 15 cases where the diameter error is minus, the length error is also minus in 9 cases, showing no evidence of diametral errors offset by length errors. In fact, they tend to accentuate each other.

DISCUSSION

The evidence secured from the analysis of a single test tree indicates that the method used gives adequately accurate results with a minimum amount of labor. This method has here been called the chosen median method of sampling the stratified twig material composing the crown of a tree.

The determination of leaf area so far has been considered as though it was merely a determination of leaf numbers. Practically considered, this is true. In any group of twigs this is the big variable—length and cross section of needles are very uniform. Thus, in the chosen median twig, after the fascicles have been counted, determining the mean needle with high accuracy by any one of a number of methods is a simple matter.

Perhaps the most satisfactory means used by the writer was to select at random a sample of needles equal to about 10 per cent of the total. In these groups of from 2 to 40 needles (test tree) the fascicle of average length was selected by inspection. It was then sectioned in the middle with a razor blade, and the sections were mounted in water under a compound microscope with measuring eye piece. The length of one of the straight sides of the needle was then determined by averaging several of the sections. The perimeter of cross section, of the ponderosa pine needles, which are three to a fascicle, was computed on the basis that the cross section of each represents one third of a circle

whose radius is the measured straight side of the needle. Needle perimeter (P) in terms of the radius is therefore :

$$P = 2 R + \frac{2 \pi R}{3} \text{ or } 4.09 R$$

and needle cross-sectional area (A) is :

$$A = \frac{\pi R^2}{3} \text{ or } 1.05 R^2$$

Total area equals perimeter times length; volume equals cross-sectional area times length. Both formulas disregard taper of the needle which involves a small section toward the tip of the leaf. More complex formulas, taking this into consideration, may be derived without much difficulty if the additional accuracy appears warranted.

The determination of the wood increment of the leafy twigs, from the evidence of the test tree, can be determined with less than 5 per cent error. The chief causes of error to be guarded against are by far the most important in the large fast-growing branches of the upper crown. Here, where there is great variance and strong skew, careful stratification is required and the recognition of a "very large" class is usually advisable. The development of homogeneous classes is usually perfectly feasible by the use of good judgment. Single unconformable twigs may advantageously be handled singly.

The experience with the test tree suggests several other minor modifications to increase accuracy. Stratification classes in which the number of twigs is very small may lead to large errors. This can happen because no single twig appears to be average, particularly when both average leaf numbers and average wood increment are sought in combination. Four remedies are suggested :

1. Instead of trying to select an unsatisfactory median twig, record the class *in toto* without recourse to sampling.
2. Select one twig of average foliage development and another of apparently average wood increment.
3. Combine with another class to make larger numbers from which selection may be made.
4. Stratify the class still further.

The first alternative is absolutely safe, but requires more work. The second is probably both safe and economical, although it lacks any field test as yet. The third appears dangerous as it increases variance. The fourth may be quite feasible.

Even in the classes with larger numbers of twigs, the endeavor to select a twig that will be average in respect to both leaves and wood increment seems frequently to have been a cause of considerable error. It would probably increase accuracy, without increasing the work involved, if two median twigs were selected—one for average leaf numbers, the other for average development of wood. It is not impossible that the same twig might satisfy both requirements, but this does not appear to happen many times.

The problem of leafless branches was not studied in the test tree. In small trees, they may be handled individually as there are not many of them. In

larger trees, they may be stratified as suggested, and sampled by the selected median twig method as in leafy twigs, with one precaution. This material is of varying ages—it has not been stratified by age as have leafy twigs. The selected median twig should therefore be carefully considered from the standpoint of diameter and width of the last ring, especially width of the last ring. Errors in the branch-wood increment are not of paramount importance when the tree increment is concerned, for branch growth comprises only about 20 per cent of the total. In computing wood increment, therefore, the greatest care must be exercised in the analysis of the bole.

It should be marked off into short "logs" from 50 to 100 cm long so arranged that knots, swellings, and injuries at the middle point of the log may be avoided. If this is done, normal diameters and ring widths may be secured. The volume is most easily computed by the Huber formula by which log volume is considered equal to the product of the cross-sectional area of the log at its midlength times its length. The logs should be sawed at their midpoint by cuts perpendicular to the axis of the bole, and great care should be taken to measure the true average diameter this year and a year ago, as great volume errors may be introduced by small errors in diameter measurements in the butt logs.

The method outlined has been applied only to trees less than 30 feet in height and 9 inches in diameter. In them, the amount of labor per tree is not excessive. As the trees grow larger, there are no greater numbers of segregated stratification groups (normally 27), but the number of twigs in each group increases and the labor of clipping and of counting, stratification, and determination of the chosen median twig increases considerably.

It is suggested that in large trees, with regularly developed crowns and whorls of branches, a single average branch may be cut at each node, instead of the whole whorl (normally 5 branches in pines). This would reduce the labor of clipping and segregation by four fifths, probably without any dangerous loss of accuracy. No test of this method has yet been made; it is merely a suggestion.

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THE VOGES-PROSKAUER REACTION AND DIFFERENTIATION OF THE COLIFORM BACTERIA¹

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THE USE of the coliform bacteria—*Escherichia* and *Aerobacter*—as indicators of the sanitary quality of water and other foods, food-production plants, and eating establishments, has been advocated for many years. Furthermore, the importance of these bacteria as agents of spoilage of such dissimilar food products as candy, cheese, cucumber pickles, dehydrated vegetables, maple sirup, olives, and potassium bitartrate (cream of tartar), has been recognized. It has, therefore, become increasingly necessary to distinguish between the two genera of coliform bacteria, especially when confronted with a problem which may involve certain aspects of sanitation as well as spoilage of a food.

Primary differentiation between the genera *Escherichia* and *Aerobacter* has been made on the basis of the Voges-Proskauer test, a qualitative measure of acetoin production from glucose. The species of *Aerobacter* (largely of nonfecal origin) produce acetoin, whereas the species of *Escherichia* (commonly used as indicators of fecal pollution) do not, under controlled and standardized conditions.

Conflicting data and opinions concerning the Voges-Proskauer reaction and differentiation of the coliform bacteria have gradually accumulated during the past decade. The disagreement has arisen especially with respect to the efficacy of various techniques and reagents for performing the Voges-Proskauer test; and the effect of such factors as the pH value of the medium and the concentration of various constituents in the medium on the production of acetoin (acetyl-methyl-carbinol) by coliform bacteria.

This investigation was undertaken to:

1. Evaluate newer techniques for determining the Voges-Proskauer reaction.
2. Ascertain the effect of physical factors, including pH value of the medium and air supply.
3. Determine the effect of concentration of glucose and other chemicals on the production of positive Voges-Proskauer reactions by coliform bacteria.

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TABLE 1
RELATIVE SENSITIVITY OF SEVERAL REAGENTS FOR THE VOGES-PROSKAUER TEST

Reagent	Concentration of acetoin in Difco M.R.-V.-P. medium, mg per liter										Approx. time for maximum color development	Acetoin in last reaction mixture with positive V.-P., p.p.m.
	100	75	50	25	20	10	5	4	3	2	1	0
	Relative sensitivity (maximum visible color development)											
Standard KOH.....	+	?	-	-	-	-	-	-	-	-	-	50
Werkman.....	+	+	?	-	-	-	-	-	-	-	-	37.1
O'Meara.....	++	++	+	+	+	?	-	-	-	-	-	10
Modified O'Meara*	+++	++	++	+	+	?	-	-	-	-	-	10
Leifson.....	+++	++	++	+	+	?	-	-	-	-	-	10.0
Barritt.....	+++	+++	+	+	+	+	-	-	1.6
Modified Barry-Smith†	+++	+++	++	+	+	+	-	-	1.65
Modified Barritt†	+++	+++	+	+	+	+	-	-	1.3

* Modified by Levine, Epstein, and Vaughn (1934).
† Formula given on p. 359.

REAGENTS FOR DETERMINING THE PRESENCE OF ACETOIN

Criteria for any efficient, routine Voges-Proskauer test require that it be sensitive, simple to prepare, easy to use, and rapid in action. All attempts to modify the original Voges and Proskauer test have striven to meet these requirements. Nevertheless, divergence of opinion concerning the merits of various modifications of the Voges-Proskauer test still persists.

Therefore, a study was made of the relative sensitivity and rate of reaction of several reagents used for the Voges-Proskauer test. These included two new reagent mixtures—modifications of the Batty-Smith and the Barritt reagents; their composition is as follows:

Modified Batty-Smith reagent: 3 ml of 5 per cent α -naphthol in absolute alcohol, 0.1 ml of 2 per cent $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$, and 1 ml of 40 per cent KOH containing 0.25 per cent creatine; used with 5 ml of test solution.

Modified Barritt reagent: 1.3 ml of α -naphthol-creatine-KOH solution containing 10 parts of 5 per cent α -naphthol in absolute alcohol and 3 parts of 40 per cent KOH containing 2.5 per cent creatine; used with 1 ml of test solution.

The relative sensitivity of the reagents was determined by testing them with redistilled acetoin contained in Difco methyl red-Voges-Proskauer medium. Observations on the eight reagents are shown in table 1.

When sensitivity alone is considered, there is evidently little choice between the Barritt (1936)⁴ test and the modifications of the Batty-Smith (1941) and Barritt tests made by the present authors. If, however, the approximate times for maximum color development are compared, the modified Barritt test is seen to be significantly better.

It is obvious that the earlier modifications are of poorer sensitivity and slower to react. Although the Leifson (1932) reagent is more sensitive than the Werkman (1930), or standard KOH reagents (American Public Health Association, 1936), the Biuret reactions which result from its use tend to obscure positive tests for acetoin.

The use of ferric chloride, either dropwise or in accurately measured amounts, also tends to obscure positive tests for acetoin. This objection is particularly valid in cases where the concentration of acetoin is barely within the limits of sensitivity of the Voges-Proskauer reagent used for its detection.

A comparison of the reaction time of the Barritt, modified Barritt, and modified Batty-Smith reagents with standard KOH and the modified O'Meara (Levine, Epstein, and Vaughn, 1934) reagent, when used to detect the production of acetoin by *Aerobacter* cultures, is shown in table 2.

The desirability of the reagents using α -naphthol (Barritt, modified Barritt, and modified Batty-Smith) is obvious. However, of the three, the modified Barritt reagent is believed to be the best. The rate of color development is consistently rapid and reaches maximum visual intensity within 1 to 5 minutes. Only one reagent is added to the culture; this fact is distinctly advantageous when many tests must be made. Furthermore, the reagent mixture is stable enough to be stored for some weeks at 0° C without impairing its reactivity, as is shown in table 3.

⁴ See "Literature Cited" for citations, referred to in the text by author and date.

Since, however, the reagent mixture does deteriorate on storage, even at 0° C, the α -naphthol and creatine-KOH solutions may be stored separately and mixed just before use. The alcoholic α -naphthol will keep for at least 2 months if stored in a tightly stoppered flask in the refrigerator, at 0° to 10°. Despite

TABLE 2
COMPARISON OF REACTION TIME OF SEVERAL REAGENTS FOR THE VOGES-PROSKAUER TESTS

Reagent	Time elapsed after addition of reagent								
	1 min.	2 min.	5 min.	10 min.	15 min.	30 min.	1 hr.	2 hrs.	4 hrs.
	Number of positive Voges-Proskauer reactions*								
Standard KOH...	0	0	0	0	0	14	15	36	41
Modified O'Meara...	0	0	0	0	26	37	45	46	46
Barritt.....	0	?†	46	47	48	48	48	48	48
Modified Barritt....	47	47	48	48	48	48	48	48	48
Modified Batty-Smith.....	?‡	47	47	48	48	48	48	49	49

* 50 cultures, including 25 strains each of *Aerobacter aerogenes* and *A. cloacae*, grown in Difco M.R.-V.-P. medium for one day at 30° C were used for the tests.

† Color not intense enough to warrant differentiation between positive and negative reactions.

‡ Presence of iron chloride causes formation of yellow color complex which interferes with detection of pink color indicative of starting positive V.-P. reaction.

TABLE 3
EFFECT OF TEMPERATURE AND STORAGE PERIOD OF α -NAPHTHOL-CREATINE-ALKALI SOLUTION ON V.-P. REACTION*

Storage period, days	Storage temperature			
	0° C	25° C	37° C	55° C
	Relative intensity of V.-P. reaction (10-minute reading)†			
1.....	++++	++++	++++	++++
2.....	++++	++++	++++	++++
3.....	++++	++++	++++	++++
4.....	++++	++++	++++	±*
7.....	++++	++ ^a	? ^a	— ^a
21.....	++++	++ ^a	? ^a	—
28.....	++++	— ^a	? ^a	—
35.....	++++	— ^a	? ^a	—
42.....	++++	— ^a	? ^a	—
49.....	++++	— ^a	? ^a	—
55.....	++++	— ^a	? ^a	—

* Test culture was *Aerobacter cloacae* grown in Difco M.R.-V.-P. medium for 1 day at 30° C.

† The reagent was prepared by mixing 3 parts of creatine-KOH solution with 10 parts of α -naphthol solution. The α -naphthol solution contained 5 grams of α -naphthol made to 100 ml with absolute ethyl alcohol. The creatine-KOH solution contained 2.5 grams of creatine in 100 ml of 40 per cent KOH. The reagent was used in the proportion of 1.3 ml of reagent to each ml of culture.

^a The V.-P. reaction was ++++ positive after 30 minutes.

previous criticisms of the keeping quality of creatine-KOH solutions, they remain stable for at least a month if stored under refrigeration at 0° to 10°.

The creatine-KOH solution especially is to be recommended. It affords the only means of accurate control of creatine added to the reaction mixture and

besides saves time and creatine. The modified Barritt and Batty-Smith reagents need only 8 mg of creatine per test as compared with the "knife-point" (approximately 25 mg) of creatine recommended for the original reagents.

On the basis of these results and considerable use in the laboratory, it is believed that the modification of the Barritt reagent made by the authors most nearly meets all requirements for an ideal Voges-Proskauer reagent for routine use. The sensitivity is equal to any Voges-Proskauer reagent now commonly used for detecting acetoin production by bacteria, and the reaction is faster. It is simple to prepare, and easier to use because only one solution is needed. It contains no chemical which tends to obscure a positive test.

TABLE 4
EFFECT OF PHOSPHATE BUFFERS OF DIFFERENT pH VALUES ON THE V.-P. REACTION

Test organism	pH 6.85*		pH 7.20*		pH 7.48*		pH 7.89*	
	2 days†	4 days†	2 days†	4 days†	2 days†	4 days†	2 days†	4 days†
	Number of positive V.-P. reactions‡							
<i>Aerobacter aerogenes</i> (25 cultures)§.	21	23	11	11	0	0	0	0
<i>A. cloacae</i> (25 cultures).....	24	24	16	20	0	0	0	0
<i>Escherichia</i> spp. (50 cultures).....	0	0	0	0	0	0	0	0

* Buffered with 0.3 M PO₄. The basal medium contained 5 grams of glucose and 7 grams of proteose peptone (Difco) per liter.

† Incubation period at 30° C.

‡ Modified Barritt reagent.

§ The *Aerobacter* cultures all were V.-P. positive in Difco M.R.-V.-P. medium.

THE EFFECT OF pH OF THE MEDIUM ON THE PRODUCTION OF ACETOIN

It is apparent from a study of the literature that the pH (reaction) of the medium must influence the production of acetoin by cultures of *Aerobacter* although the extent of effect is not clear. The results reported by Mickelson and Werkman (1938) and Banerjea (1944) indicate a definite effect of pH on production of acetoin by cultures of *A. aerogenes*, although not so striking as those reported by Silverman and Werkman (1941). The latter investigators found that cell-free enzyme prepared from a culture of *A. aerogenes* had a definite optimum pH range for acetoin production and a pH value above which its production was suppressed.

A study was made of the effect of pH on the ability of *Aerobacter aerogenes* and *A. cloacae* cultures to produce acetoin in media with various pH values controlled by the use of 0.3 M phosphate buffer or 0.1 M citric acid plus 0.2 M phosphate buffer. Results obtained with phosphate buffers are shown in table 4. The pH value of the medium does have a striking effect on the ability of *Aerobacter* cultures to produce acetoin if the pH is controlled by buffers so that the intermediate and terminal values are identical or nearly identical to the initial value. Under such conditions it is found that pH values between 7.2 and 7.3 markedly suppress acetoin production. Similar results were obtained with the citric acid-phosphate buffer. Other buffer systems either were toxic or did not control the pH of the medium adequately.

Banerjea (1944) and Smith, Gordon, and Clark (1946) have suggested that phosphate in the medium suppressed the number of positive Voges-Proskauer reactions, although phosphate as di-basic potassium phosphate is a constituent of the M.R.-V.-P. medium most widely used the world over. Others, including Reynolds and Werkman (1937), Kluyver and Molt (1939), and Sakaguchi and Tada (1940), preferred to control pH values of their media with carbonate salts. A study was therefore made of the effect of carbonate salts used to control pH, on acetoin production. The results with carbonate salts (calcium, magnesium, and zinc) also show that if the intermediate and terminal pH values are controlled at or near the initial value, the pH has an important effect on acetoin production; these results thus confirm the data obtained with phosphate and citric acid and phosphate buffers and again substantiate with growing cultures the observations of Silverman and Werkman (1941) on the effect of pH on enzymatic acetoin production.

TABLE 5
THE EFFECT OF AEROBIC AND ANAEROBIC CONDITIONS ON ABILITY OF *Aerobacter*
CULTURES TO PRODUCE POSITIVE V.-P. REACTIONS

Test organism	Aerated culture* (shaking machine)				Anaerobic culture† (anaerobic jar with nitrogen gas)			
	Number of V.-P. positive cultures‡							
	1 day	2 days	3 days	4 days	1 day	2 days	3 days	4 days
<i>Aerobacter aerogenes</i> (25 cultures) .	16	11	7	5	24	24	24	24
<i>A. cloacae</i> (25 cultures)	24	24	23	23	24	25	25	25

* 100 ml of Difco M.R.-V.-P. medium in 500-ml Erlenmeyer flask incubated at room temperature (25–30° C).

† 100 ml of Difco M.R.-V.-P. medium in 200-ml Erlenmeyer flask incubated at room temperature (25–30° C).

‡ Barritt reagent without creatine.

THE EFFECT OF AIR SUPPLY ON PRODUCTION OF ACETOIN

When continuous shaking was used to facilitate control of pH with insoluble carbonate salts it was noted that the resulting aeration suppressed acetoin production by some cultures of *Aerobacter aerogenes*. Consequently, a more detailed study was made to determine whether the effect noted was the result of aeration or some other factor. Results of one aeration experiment with Difco M.R.-V.-P. medium are shown in table 5.

It has been assumed for some years that aerobic conditions (that is, aeration) favor the production of acetoin by cultures of *Aerobacter* as claimed by O'Meara (1931), Reynolds and Werkman (1937), Mickelson and Werkman (1938) and others. Nonetheless, it is quite evident that, under the conditions of this experiment at least, aerobic conditions suppressed rather than favored the production of acetoin, particularly by cultures of *A. aerogenes*.

Several characteristic types of cultures were found on the basis of their ability to produce acetoin. These types are shown in table 6.

Further proof that aeration adversely influenced acetoin production by cultures of *Aerobacter* was obtained by use of the aeration apparatus shown

TABLE 6
REACTIONS OF VARIOUS CULTURES OF *Aerobacter* SPECIES GROWN UNDER AEROBIC
AND ANAEROBIC CONDITIONS

Species and type designation	Number of cultures	Aerobic*				Anaerobic†			
		V.-P. reaction‡ after incubation at room temperature for							
		1 day	2 days	3 days	4 days	1 day	2 days	3 days	4 days
<i>Aerobacter aerogenes</i> :									
Type A.....	8	—	—	—	—	++++	++++	++++	++++
Type B.....	6	++++	—	—	—	++++	++++	++++	++++
Type C.....	8	++++	++++	—	—	++++	++++	++++	++++
Type D.....	1	++++	++	++	+	++++	++++	++++	++++
Type E.....	1	+	++	+++	+++	++++	++++	++++	++++
Type F.....	1	—	+	+	+	+	+	+	+
<i>A. cloacae</i> :									
Type A.....	22	++++	++++	++++	++++	++++	++++	++++	++++
Type B.....	1	++++	++++	—	—	++++	++++	++++	++++
Type C.....	1	++++	—	—	—	++++	++++	++++	++++
Type D.....	1	—	++	+++	++++	+	++	++	++

* Shaken on machine to afford complete aeration.
† In anaerobe jars with atmosphere of nitrogen.
‡ Modified Barritt reagent.

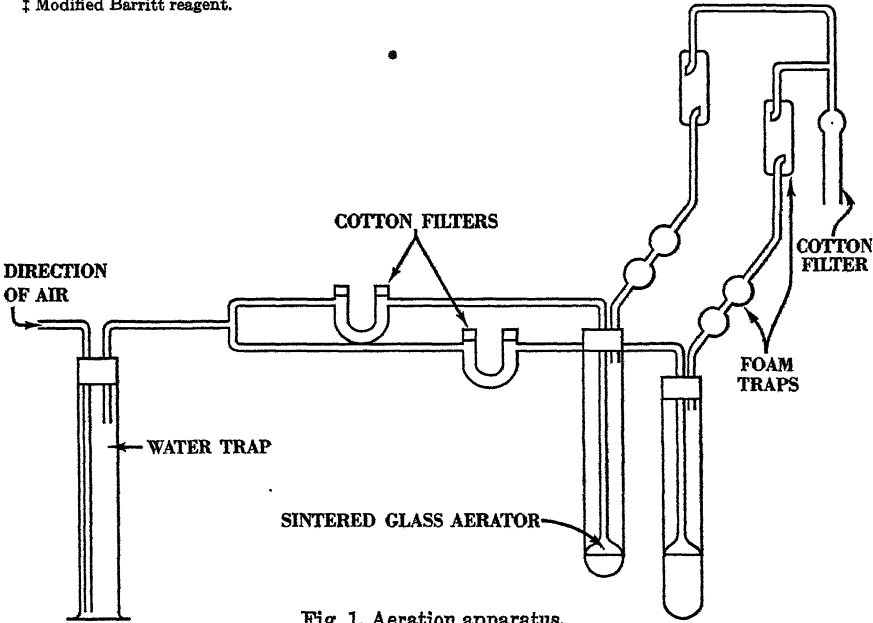


Fig. 1. Aeration apparatus.

in figure 1. In experiments with this apparatus it was possible to aerate the inoculated medium by compressed air so that all or any desired portion of the medium was continuously and vigorously aerated. Those cultures of *A. aerogenes* and *A. cloacae* that showed negative or weak Voges-Proskauer re-

TABLE 7

THE EFFECT OF CONCENTRATION OF GLUCOSE AND THE PRESENCE OF CARBONATE SALTS ON
ACETON PRODUCTION BY COLIFORM BACTERIA

Culture	O'Meara medium*	O'Meara medium minus glucose*	O'Meara medium + 2 per cent glucose*	Difco medium*	Difco medium with added glucose*					Difco medium with 2 per cent glucose and 1 per cent carbonate salt*				
					Total glucose, grams per liter					Carbonate added†				
					10	20	30	40	80	Ca	Mg	Mn	Zn	
					V.-P. reaction after 4 days at 30° C, Barritt reagent without creatine‡									
<i>Escherichia coli</i> ...	±	-	+	-	-	-	±	±	-	+	++	-	+	+
<i>E. acidilactici</i> ...	-	-	+	-	±	±	±	±	-	+	+	-	+	+
<i>E. neopollitana</i> ...	-	-	+	-	±	±	±	±	-	+	+	±	+	+
<i>E. communior</i> ...	±	-	+	-	-	-	±	±	-	+	+	-	±	±
<i>E. freundii</i>	-	-	+	-	-	±	±	±	-	+	+	-	±	±
<i>E. intermedium</i> ...	-	-	+	-	±	±	±	±	-	+	+	-	±	±
<i>Aerobacter aerogenes</i> ...	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
<i>A. cloacae</i> ...	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Control.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* All media dispensed in 100-ml portions in 300-ml Erlenmeyer flasks and sterilized at 15 pounds' steam pressure for 15 minutes. With media having more than 2 per cent glucose, the sugar was sterilized separately and added aseptically.

† Sodium bicarbonate and the carbonate salts of barium, bismuth, cadmium, iron, and lead gave results similar to calcium and zinc carbonates.

‡ ± indicates weak but definite, persistent pink; +, ++, +++ indicate relative intensity of reaction.

actions when continuously and completely aerated by shaking showed negative reactions when completely aerated with this apparatus. These same cultures showed strongly positive reactions when only partially aerated. Other cultures whose reaction was not affected by aeration by shaking were not influenced by vigorous and complete aeration with this apparatus.

Although complete explanation of the effect of aeration on acetoin production must await final quantitative studies, it is believed that the striking effect of aeration on some cultures and not others is the result of their differences in ability to decompose acetoin. It is well known that growing cultures of coliform bacteria decompose acetoin, as has been shown by Paine (1927), Williams and Morrow (1928), and Tittsler (1938), although it is not clear whether decomposition was effected under aerobic or anaerobic conditions. However, more recently Stanier and Fratkin (1944) have shown that acetoin is oxidized by cell suspensions of one strain of *A. aerogenes*. (These observations should not obscure the fact that aerobic conditions are absolutely necessary for the development of the color reaction of the chemical test for acetoin.)

FACTORS AFFECTING ACETOIN PRODUCTION BY SPECIES OF *ESCHERICHIA*

Some confusion exists concerning the ability of *Escherichia coli* and related species to produce acetoin as indicated by positive Voges-Proskauer reactions. A few (O'Meara, 1931; Reynolds and Werkman, 1937; Kluyver and Molt, 1939; and Sakaguchi and Tada, 1940) have demonstrated the production of positive Voges-Proskauer reactions by species of *Escherichia*, but an overwhelming majority of others have not been so successful.

A study of the literature reveals that workers who demonstrated acetoin production by *Escherichia coli* and related species used media significantly different from those prescribed as "standard" by the American Public Health Association (1936) and the Ministry of Health (1939). O'Meara (1931) used a synthetic medium which contained 1 per cent sodium fumarate in addition to the customary 0.5 per cent glucose. Other media used successfully for demonstration of acetoin production (positive Voges-Proskauer reactions) by species of *Escherichia* commonly contained four to eight times the recommended concentration of glucose and from 1 to 2 per cent of sodium bicarbonate or calcium carbonate in addition to other salts not specified as constituents of the standard medium.

It was realized that the composition of the medium might have as marked an effect on the production of positive Voges-Proskauer reactions by species of *Escherichia* as do the constituents of the medium on hydrogen sulfide production by these bacteria, as previously shown by Vaughn and Levine (1936). Therefore experiments were conducted to determine the effects of concentration of glucose and the use of several insoluble carbonate salts on the production of acetoin by cultures of *Escherichia*. Results are shown in table 7.

The concentration of glucose, the presence of various carbonate salts and fumarate (O'Meara media) do have an effect on the ability of species of *Escherichia* to produce positive Voges-Proskauer reactions. Forced aeration of the growing cultures was not necessary in order to demonstrate positive

Voges-Proskauer reactions by the test cultures, although it was so specified by Reynolds and Werkman (1937), and Kluver and Molt (1939).

Since the constituents of the standard medium do not include carbonate salts or fumarate and the glucose concentration is specified as 5 grams per liter, it is quite apparent that the conflicting views concerning the production of positive or negative Voges-Proskauer reactions by *Escherichia* cultures result primarily from variations from the "standard" both in composition and concentration of constituents of the medium used by those observing positive Voges-Proskauer reactions with *Escherichia* cultures.

DISCUSSION

It is evident that the genera *Escherichia* and *Aerobacter* are more closely related than once realized because it is true that *E. coli* and related species do produce acetoin (positive Voges-Proskauer reactions) in certain culture media. It is equally true that cultures of *Aerobacter* fail to produce acetoin in culture media under certain conditions. However, it is apparent from the data presented here and elsewhere that the concentration of acetoin produced by cultures of *Escherichia* is small and related in some manner to the amount of glucose and the presence of other compounds, as carbonates and fumarate in the medium. Furthermore, if "standard" media are used, the amount of acetoin produced by species of *Escherichia* generally is not detectable qualitatively, even with the very sensitive α -naphthol Voges-Proskauer reagents, without first subjecting the medium to chemical treatment and distillation.

Therefore, the demonstration that members of the genus *Escherichia* do produce acetoin under certain conditions does not detract from the value of the Voges-Proskauer test if performed under standardized conditions.

A similar situation exists with respect to the ability of coliform bacteria to produce hydrogen sulfide. As shown by Vaughn and Levine (1936), the majority of the coliform bacteria produce H_2S from proteose peptone (Difco) in a liquid medium. Yet if the medium contains 1.5 per cent agar in addition to proteose peptone and ferric citrate (Levine, Vaughn *et al.*, 1932), only cultures of *Escherichia freundii* produce positive H_2S reactions and so the medium can be used for differential purposes.

SUMMARY AND RECOMMENDATIONS

The results described here have shown that the reagents used for determining the Voges-Proskauer test vary widely in sensitivity and speed of reaction; that the pH of the medium, if controlled by buffers, does materially influence the ability of cultures of *Aerobacter* to produce acetoin; that aeration hinders rather than favors acetoin production by cultures of *A. aerogenes*, whereas aerobic or anaerobic conditions have little effect on acetoin production by the majority of the cultures of *A. cloacae*; and finally, that the concentration of constituents and the composition of the medium markedly influence the ability of cultures to produce positive Voges-Proskauer reactions.

As a result of these investigations and other knowledge which has accumulated in the literature, the following recommendations are made for determining the Voges-Proskauer reaction of coliform bacteria:

1. The media for determining the Voges-Proskauer reaction should be prepared and used as specified by the American Public Health Association.

2. The α -naphthol, creatine, KOH solution—a modification of the Barritt reagents made by the authors—is recommended for determining the Voges-Proskauer reaction.

3. The inoculated medium should be incubated at 30° C.

4. Excessive aeration of the growing cultures must be avoided.

5. Daily tests for acetoin production must be made. Although the majority of *Aerobacter* cultures produce positive Voges-Proskauer reactions after 1 day of incubation, some cultures give positive Voges-Proskauer reactions only after 2 days of incubation and others show positive reactions only on the third or fourth day of incubation. Because of this it is advantageous to use 1 ml of culture for conducting the Voges-Proskauer test.

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PROPAGATION OF THE ORIENTAL FRUIT MOTH UNDER CENTRAL CALIFORNIA CONDITIONS¹

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INTRODUCTION

SINCE 1943 the State Department of Agriculture has discovered low populations of the Oriental fruit moth, *Grapholitha molesta* Busck, in several widely scattered peach-growing localities in central California. The San Joaquin and Sacramento valleys which comprise this area contain a large proportion of all peach plantings within the state. These valleys have a different climate from that encountered by the Oriental fruit moth in other important peach-producing states. The very hot, dry summers and mild, foggy winters, the extensive practice of irrigation, and the very long growing season—averaging 289 days in the vicinity of Fresno (Bonnett, 1941)⁴—are environmental factors which may affect the future development of the Oriental fruit moth populations.

In the course of a general study of the life history of the Oriental fruit moth in central California, a number of experiments were performed in the insectary at Dinuba to determine whether or not the hot summer climate of the central-valley area retards its propagation; and, if so, whether it is retarded enough to reduce the potentiality of the moth as a pest. It is the purpose of this report to describe this phase of the life-history work, and to present other observations on mating and oviposition which have a bearing on the dispersal and build-up in thinly distributed populations of Oriental fruit moths such as now occur in this part of the state.

METHODS AND STOCKS

The moths were reared in a screened insectary and subjected as nearly as possible to outdoor conditions. The cages and other receptacles were constructed of light materials, thin sheet plastic and cheesecloth or gauze, in order to minimize the time required for their interiors to come into equilibrium with atmospheric conditions during rapid weather changes.

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⁴ See "Literature Cited" for citations, referred to in text by author and date.

When Oriental fruit moths from several localities were compared in side-by-side laying tests, it became apparent that all were not equally prolific. They were then given stock designations according to the locality from which the larvae were taken. The original insectary moths were reared from larvae obtained from a peach orchard in Tulare County; they were designated as the Dinuba stock. The moths herein designated as the southern California stock were obtained later from orchards in Orange County. Two other stocks were developed from larvae taken in Fresno County, in central California. They are subsequently referred to as the Parlier I and Parlier II stocks. The Parlier I stock was started from a pair of moths, Parlier ♀ × Dinuba ♂.

MATING HABITS

Oriental fruit moths usually emerged from their cocoons in the morning on sunny days, between 7 a.m. and 11 a.m. Individuals of both sexes were sometimes capable of mating on the day of emergence, though ordinarily the first successful matings were not made until the second or third day of adult life. Casual observations of copulating pairs indicate that mating occurs in the evening, after 5 p.m., which coincides with the period of greatest flight activity and oviposition by older individuals. During the cool spring months, copulating pairs were sometimes seen in the early afternoon.

The number of successful matings which males are capable of making in one overnight period was experimentally determined for six males. Each of six 2-day-old unmated males was placed in a small cage with five 2-day-old females (1st to 5th in table 1) and allowed to remain in the cage overnight. On the following morning the males were discarded and the thirty females from the six cages were placed each in a separate cage and left for 10 days. At the end of this period the number of eggs laid by each female and the number which later hatched were recorded. The numbers of eggs deposited are given by the upper figures, and the numbers hatched by the lower figures in each cell of table 1.

Of the six males tested, three (nos. 3, 5, and 6) apparently mated with but one female each. The latter deposited average broods of viable eggs. The other three males (nos. 1, 2, and 4) apparently attempted to mate with more than one female but only one female from each cage produced a normal brood. The unsuccessfully mated females laid nonviable eggs. The numbers of these eggs were, with one exception, greater than expected from unmated females. Unmated females occasionally laid one or two eggs during their lives but none of the eggs were ever observed to hatch. The average brood expected for mated females of the stock used is approximately 114 eggs, over 85 per cent of which are viable when deposited on wax paper.

Females mated successfully during a brief association (12 hours) with males appeared to lay as many eggs as those kept with males throughout the laying period.

Males may retain their fecundity throughout life. This was demonstrated by an experiment in which the same males were mated, on successive days, to different young females (table 2). The results are shown as the number of eggs laid in ten days (upper figure) by each of the test females, and the number of eggs which later hatched (lower figure).

Five of the males (A to E) were placed with the first females on the day of emergence and with different females on each of the five succeeding days. All of the test females were less than 12 hours of age when presented to the males. Successful matings did not occur until the males were at least 3 days of age (males B and C). One male (A) was unable to make a fertile union with any of the six females presented on different days, possibly for the reason

TABLE 1
RESULTS OF TESTS TO DETERMINE THE NUMBER OF SUCCESSFUL MATINGS
MADE BY MALES DURING ONE 12-HOUR (OVERNIGHT) PERIOD

Designation of males	Oviposition record for each of the 30 test females				
	Number of eggs laid				
	Number of eggs hatched				
	1st	2nd	3rd	4th	5th
1	0	$\frac{102}{89}$	0	$\frac{10}{0}$	$\frac{18}{0}$
2	$\frac{147}{143}$	$\frac{4}{0}$	0	$\frac{5}{0}$	0
3	$\frac{139}{129}$	0	0	0	0
4	$\frac{1}{0}$	$\frac{3}{0}$	0	0	$\frac{120}{115}$
5	0	0	0	0	$\frac{141}{132}$
6	0	0	0	$\frac{167}{163}$	0

that the females were not ready for mating during their first 12 hours as adults.

The procedure was slightly modified for a second group of five males (F to J). They were presented to the first of the females on the day after emergence; and all of the test females were 24 hours old when placed with them. Two of the males (G and H) mated on the second day, and all mated on the third day. This series was discontinued after 5 days.

For a third group of five males (K to O), the mating trials with females 24 hours old were started on the day after emergence and continued for 15 days, or until the first male died. Males K to O mated irregularly, although the number of failures occurred with greater frequency after the first 8 days of life. Forty-two of the seventy-five females with which they were tested laid fertile eggs, and twelve were induced to lay more than two infertile eggs. The data of the long series suggest that there may be a decline of mating capacity in males of advanced age.

A limited number of tests were made to find out whether or not moths of both sexes are able to mate for the first time in later life. Young females, 24 hours of age, were found to be capable of laying viable eggs when mated to previously unmated males 3 to 10 days of age; and, in reciprocal tests, pre-

TABLE 2
THE NUMBERS OF EGGS DEPOSITED IN TEN DAYS BY YOUNG FEMALES WHEN MATED TO
MALES OF DIFFERENT AGES

Age of males in days	Males first mated on day of emergence					Males first mated on day after emergence												
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O			
	Oviposition records for each of the test females																	
	Number of eggs laid																	
	Number of eggs hatched																	
1.....	$\frac{4}{0}$	0	0	0	$\frac{4}{0}$			
2.. ..	$\frac{25}{0}$	$\frac{7}{0}$	0	$\frac{1}{0}$	$\frac{4}{0}$	0	$\frac{15}{11}$	$\frac{100}{92}$	$\frac{1}{0}$	$\frac{1}{0}$	$\frac{116}{110}$	$\frac{148}{139}$	$\frac{112}{106}$	$\frac{128}{127}$	$\frac{2}{0}$			
3.....	0	$\frac{112}{108}$	$\frac{101}{96}$	0	0	$\frac{171}{161}$	$\frac{146}{110}$	$\frac{150}{147}$	$\frac{141}{136}$	$\frac{140}{136}$	0	$\frac{186}{177}$	0	0	$\frac{7}{0}$			
4 ...	$\frac{29}{0}$	0	$\frac{133}{126}$	$\frac{18}{0}$	$\frac{140}{120}$	0	$\frac{55}{49}$	$\frac{94}{90}$	$\frac{88}{87}$	$\frac{4}{1}$	$\frac{126}{100}$	$\frac{153}{148}$	0	$\frac{158}{149}$	$\frac{171}{159}$			
5	$\frac{7}{0}$	$\frac{112}{111}$	$\frac{218}{198}$	$\frac{146}{129}$	$\frac{191}{164}$	2	$\frac{87}{83}$	$\frac{134}{126}$	$\frac{127}{125}$	$\frac{118}{116}$	9	$\frac{187}{166}$	$\frac{188}{186}$	$\frac{163}{168}$	$\frac{119}{108}$			
6....	0	$\frac{177}{158}$	0	$\frac{119}{101}$	$\frac{3}{0}$..	$\frac{132}{124}$	0	$\frac{206}{198}$	$\frac{187}{185}$	0	$\frac{182}{166}$	$\frac{145}{118}$	$\frac{115}{99}$	$\frac{112}{92}$			
7..	$\frac{78}{70}$	$\frac{27}{26}$	$\frac{94}{92}$	$\frac{167}{157}$	0			
8..	$\frac{7}{0}$	$\frac{104}{102}$	$\frac{89}{82}$	$\frac{65}{64}$	$\frac{53}{46}$			
9.....	$\frac{18}{16}$	$\frac{3}{0}$	$\frac{101}{83}$	$\frac{92}{91}$	$\frac{12}{10}$			
10..	$\frac{39}{36}$	$\frac{3}{0}$	$\frac{3}{0}$	$\frac{13}{4}$	0			
11.....	0	$\frac{7}{0}$	0	0	$\frac{2}{0}$			
12.....	0	$\frac{199}{198}$	$\frac{175}{168}$	$\frac{141}{137}$	$\frac{126}{124}$			
13.....	0	$\frac{18}{12}$	0	$\frac{2}{0}$	$\frac{40}{15}$			
14.....	$\frac{1}{0}$	$\frac{64}{55}$	0	$\frac{28}{13}$	0			
15.....	$\frac{7}{0}$	$\frac{25}{0}$	$\frac{151}{125}$	$\frac{166}{157}$	$\frac{2}{0}$			
16.....	$\frac{7}{0}$	$\frac{115}{86}$	0	$\frac{3}{0}$	$\frac{25}{0}$			

viously unmated females 3 to 10 days of age mated successfully with males 24 hours of age. Previously unmated males and females, up to 10 days of age, produced broods of viable eggs when mated together. Quantitative data are not available at the present time for matings of this type.

OVIPOSITION

General Laying Habits. Oviposition was observed to begin on the second day after emergence, or later, according to conditions of sunlight and temperature. Mating often occurred on the evening of the day of emergence in midsummer, and the first eggs frequently appeared 24 hours later. Females were not observed to lay on the day of the initial mating. The duration of the true preovipositional period was more commonly 3 to 5 days, with periods ranging up to 14 days in the cool weather of early spring.

On warm, sunny days the peak of oviposition occurred between the time of sunset and full darkness. Very few eggs were laid in the early morning hours and none were ever observed to be laid during the day. These observations on the time of laying at Dinuba agree generally with those made by Stearns and Neiswander (1930) on the Oriental fruit moth at Wooster, Ohio. They noted that, in July, oviposition usually started between 4 and 5 p.m. and continued until about midnight, and that approximately 90 per cent of the eggs were deposited between 6 and 9 p.m.

The temperature range within which oviposition is possible could not be determined precisely by the methods used in this work. According to tests performed at Dinuba during 1946, there was no obvious decline in oviposition at the peak summer temperatures recorded in the insectary (table 5, page 376). The highest maximum temperature recorded during oviposition tests was 104° F. The lowest 1946 temperatures recorded during the tests occurred on October 4, 5, and 6, during which time 100 females of the southern California stock averaged 25.6 eggs a day each. The insectary temperatures for the three days averaged 68.3° at sunset (approximately 5:37 p.m.) and 61.7° at 7:00 p.m. Others have noted that oviposition declines when the temperature falls below 70° at sunset (McConnell, 1934) and that practically no eggs are laid when the temperature during the normal egg-deposition period is below 60° (Peterson and Haussler, 1930). A minimum temperature of 55° is given by Cagle (1930).

The ovipositional period averaged 10.1 days during 1945, with 19 days as the longest noted for any of the insectary strains. The maximum number of eggs generally appeared on one of the first 3 days of the laying period. The largest number of eggs deposited by a single female on the first laying day was 45, which was also the maximum observed for any day during oviposition. After beginning abruptly, the trend of oviposition varied considerably during relatively uniform weather conditions. Some females continued to oviposit at a reduced rate after the peak, producing 8 to 15 eggs daily until near the end of the laying period. Others oviposited heavily during the first half of the laying period but continued thereafter with only a small number of eggs each day. Many laid haphazardly throughout, and a few moths of the Dinuba stock did not lay at all.

During periods of low temperatures, the reproductive life of females was

prolonged and the eggs were distributed more evenly throughout the laying period.

The most prolific of the stocks laid an average of 114.1 eggs per female during 1946, and the maximum number laid by any female under observation was 234.

A composite daily production record for 119 females, each one placed in a small cage with three males, is given in table 3 to show how oviposition

TABLE 3
OVIPOSITION IN RELATION TO AGE OF FEMALES
DINUBA STOCK, 1945

	Spring generation	First generation	Second generation	Third generation	Total or average
Ovipositing period	Apr. 22-May 31	June 13-July 23	July 15-Aug. 22	Aug. 13-Oct. 3	
Number of females	19	36	33	31	119
Total eggs laid	913	1,597	1,313	1,605	5,428
Age of females, days	Cumulative per cent of eggs laid				
2	1.0	1.5	8.2	1.3	3.0
3	6.4	11.8	12.6	11.1	10.9
4	22.8	22.3	20.3	21.5	21.8
5	43.8	31.6	27.1	33.0	33.0
6	58.3	44.0	42.7	38.4	44.5
7	73.0	53.1	51.8	46.5	54.3
8	79.8	61.4	64.6	59.5	64.7
9	87.3	71.1	71.8	66.9	72.8
10	90.7	77.2	80.3	76.7	80.0
11	92.9	84.5	87.5	81.4	83.9
12	96.0	88.5	92.7	86.3	90.0
13	97.3	92.5	96.6	92.0	94.1
14	98.2	96.6	98.6	94.5	96.7
15	98.7	98.2	99.5	96.9	98.2
16	98.8	99.0	99.5	97.6	98.8
17...	99.2	99.3	99.9	99.4	99.5
18...	99.9	99.8	100.0	99.9	99.8
19...	100.0	100.0	.	99.9	99.9
20...	100.0	100.0

varied according to age of the females. The build-up in numbers of eggs deposited by the moths as a group was not so rapid as for individuals because of variations in the length of the preovipositional periods of the females comprising the group. Four generations of females are shown to have laid approximately two thirds of their eggs during the first 8 days of adult life. Later work indicates that the total number of eggs deposited by this group of females was below average—probably because of the type of food on which the larvae were reared—but that the trend of laying was normal.

Variations in the number of eggs laid by individual females are shown by the frequency distributions in table 4.

Oviposition under Different Weather Conditions. In order to compare numbers of eggs deposited per female under different weather conditions, ovipositional tests were made at intervals between April 18 and September 9, 1946.

Freshly emerged females were counted into one or more cages and provided with an excessive number of males, in the approximate ratio of 65 males to 50 females. Possible crowding effects were minimized by placing not more than 50 females, or 115 moths of both sexes, in one cage. When the number of females available was not an even multiple of 50, they were equally distributed in two or more cages. The cages used for this purpose were thin-walled plastic tubes, $4\frac{1}{2}$ inches in diameter and 6 inches long, each covered on one end with surgical gauze and on the other end with bleached muslin. The use of the muslin on the end placed nearer to the light source reduced flight

TABLE 4
VARIATION IN THE NUMBERS OF EGGS LAID BY FEMALES OF TWO STOCKS, 1946

Strain	Number of females observed	Number of eggs laid												
		0	1-20	21-40	41-60	61-80	81-100	101-120	121-140	141-160	161-180	181-200	201-220	221-240
		Per cent of females observed												
Dinuba	133	12.8	14.3	7.5	11.3	13.5	15.8	10.5	9.8	2.2	1.5	. .	0.8	.
Southern California	132	5.3	2.3	11.4	12.9	10.6	14.4	12.1	8.3	12.1	5.3	3.8	1.5

injuries to a very low level. In fact, after the techniques of transferring moths from cage to cage were perfected, the number lost by accident proved to be negligible. A lidlike arrangement at one end of the cage facilitated the changing of the wax-paper lining on which the eggs were deposited. A compressed pad of wet cotton in a Syracuse dish served as the drinking fountain and humidifier. The cages filled with moths were suspended on wood frames adjacent to the south screen wall of the insectary, where they received diffuse sunlight during the early morning and late afternoon.

Earlier experience with Oriental fruit moth oviposition showed that the variations in numbers of eggs laid by small, homologous samples of concurrently laying moths were apt to be greater for short periods of time than for long periods of time. Consequently, a 7-day time unit was adopted as a compromise between highly variable production under uniform weather conditions during 1- or 2-day test periods, and more uniform production but highly variable weather conditions during long, overlapping test periods. However, for estimating total laying capacity, seven of the twenty-one tests were run to completion—that is, for the entire laying period.

The average number of eggs produced per female in 7 days and the average temperatures during the test periods are given in table 5. The data show that oviposition did not diminish to any great extent during periods of highest average temperatures. In fact, for the southern California and Dinuba stocks, the highest averages were obtained during periods having the highest average temperatures. The averages obtained for small samples of Parlier I moths do not show a well-defined trend. Except for low averages in June, these moths oviposited about as well in hot weather as in cool weather. In five out of six tests made during July and August with Parlier I moths, the averages were well above the weighted mean for the series of tests.

All of the moths used in the tests were derived from larvae fed on green peaches, the quality of which varied according to the size of the fruit. The moths used in the tests during April are shown to have deposited more eggs than those tested in June (table 5). These were spring-generation moths derived from larvae fed on large green peaches (Miller's Late variety) during

TABLE 5
AVERAGE NUMBER OF EGGS DEPOSITED PER FEMALE DURING THE FIRST SEVEN DAYS OF THE OVIPOSITIONAL PERIOD

Initial laying date (1946)	Average temperatures for 7-day periods		Stocks tested					
			Southern California		Dinuba		Parlier I	
	Daily mean	At 7:00 p.m.	Number of females	Average number eggs per female	Number of females	Average number eggs per female	Number of females	Average number eggs per female
Apr. 18	66.6	75.4	54	41.1	60	42.0	10	57.8
Apr. 20	68.3	76.4	74	59.2	125	50.1	35	68.7
Apr. 24	67.9	75.9	40	57.7	150	40.8	23	54.5
June 6	68.3	78.6	110	37.7	72	17.4	90	21.7
June 8	69.9	79.7	96	38.0	97	27.0	83	29.0
June 11	71.6	82.0	111	65.2	120	33.7	50	25.8
June 22	72.8	81.7	.	.	110	28.3	.	.
June 25	75.9	84.7	.	.	100	43.2	.	.
June 27	77.3	85.9	.	.	100	48.2	.	.
June 29	78.8	88.3	.	.	150	32.3	.	.
July 10	79.7	89.7	250	102.8	111	56.7	100	57.9
July 12	79.1	89.3	150	92.6	80	37.8	100	60.1
July 15	81.6	90.1	150	101.9	75	56.2	89	52.2
July 26	79.5	88.7	.	.	135	69.9	.	.
July 30	81.4	90.7	135	97.2
Aug. 6	80.8	90.3	150	106.0	75	59.8	90	38.1
Aug. 8	81.3	90.1	120	88.6	200	38.7	50	52.6
Aug. 10	80.1	88.3	150	82.9	200	33.7	100	49.1
Aug. 23	76.6	83.9	100	91.4	200	44.7	.	.
Aug. 25	74.5	81.1	77	96.4	200	49.6	.	.
Sept. 27	70.6	72.4	100	81.5
Total or weighted mean...	.	.	1,867	83.4	2,380	42.7	820	45.5

the fall of 1945. The low average made by first-generation moths in June was probably due to the fact that very small, prethinning peaches were fed to the first batches of 1946 larvae. Because the small peaches tended to dry out, the larvae were obliged to transfer to new ones added from time to time. Therefore the increase in production, beginning in late June and continuing through July, may be attributed to rising temperatures, to the increasing size of the peaches provided as food for the larvae, or to both factors.

Oviposition by Moths of Different Stocks. Averages of the numbers of eggs deposited per female during complete ovipositional periods are listed in table 6. The weighted means of 114.1 eggs per female for moths of the southern California stock and 56.5 eggs per female for those of the Dinuba stock are assumed to differ significantly, since the ratio between them is approximately 2:1. On the other hand, the difference between the mean number of eggs pro-

duced by *Dinuba* and *Parlier I* moths is possibly not significant, especially in view of the small differences obtained in the 7-day tests.

Another stock, *Parlier II*, was introduced into the insectary during 1946. In one test made near the end of the season, 74 *Parlier II* females laid an average of 81.7 eggs as compared with an average of 143.0 eggs per female for southern California females laying on the same dates (table 6). The ratio of the averages for *Parlier II* and southern California moths in this one test ($81.7/143 = 0.57$) agrees closely with the ratio between the means obtained

TABLE 6
AVERAGE NUMBER OF EGGS DEPOSITED PER FEMALE DURING THE ENTIRE
OVIPOSITIONAL PERIOD

Initial laying date (1946)	Average temperatures during oviposition		Stocks tested							
			Southern California		<i>Dinuba</i>		<i>Parlier I</i>		<i>Parlier II</i>	
	Daily mean	At 7:00 p.m.	Num- ber of females	Average number eggs per female	Num- ber of females	Average number eggs per female	Num- ber of females	Average number eggs per female	Num- ber of females	Average number eggs per female
Apr. 20	87.3	75.9	74	62.9	125	62.9	35	74.3
June 11.	73.6	84.0	111	84.0	120	46.2	50	48.1
June 29.	79.6	89.7		..	150	47.2
July 10.	81.9	91.2	250	121.3	111	76.8	100	79.8	.	.
Aug. 10.	80.0	88.3	150	104.0	200	48.8	100	68.7	.	..
Aug. 29.	71.7	77.5	199	134.1	150	63.8
Sept. 27.	67.2	70.8	100	143.0	74	81.7
Total or weighted mean..	884	114.1	856	56.5	285	69.7

for *Parlier I* and southern California moths ($69.7/114.1 = 0.61$). It therefore appears that the two *Parlier* stocks have similar laying tendencies.

A series of comparative tests were made with two stocks to determine how averages of the numbers of eggs laid are affected by infertile or otherwise unproductive females. For each test, 15 females of the *Dinuba* stock and 15 females of the southern California stock were placed, individually, in small cages containing 3 males. The cages were observed for 10 days, and separate records of eggs laid and hatched were kept for every female. In the nine tests summarized in table 7, the two stocks proved to be unlike in regard to the number of unproductive females observed. Every one of the southern California females laid eggs, whereas approximately 12.8 per cent of the *Dinuba* females failed to do so. The hatching records revealed deficiencies of another type. There were a few females that deposited only nonviable eggs. Presumably the latter were infertile, for embryos did not develop. Approximately 11.2 per cent of the *Dinuba* females and 4.5 per cent of the southern California females belonged in this category.

Estimates of the frequency of unproductive females, as furnished by this series of tests, provide a means of adjusting ovipositional averages to exclude the nonreproductive individuals. For example, the 113 *Dinuba* females deposited 8,712 eggs, or an average of 65.6 eggs per female for individuals of all categories, or 79.3 eggs per female for laying individuals. By further eliminat-

ing the 15 defectives of the second category, together with their total production of 113 eggs, the average is increased to 84.2 eggs per female for fertile females. Similarly, the 132 southern California females deposited 14,864 eggs to average 112.6 eggs per female for laying females. The 6 defectives laid 196 eggs, hence the average for the fertile females only was 116.4 eggs per female.

Although the unproductive females occurred with greater frequency in the less vigorously reproducing Dinuba stock, the differential effect of this phe-

TABLE 7
SUMMARY OF 10-DAY OVIPOSITIONAL TESTS TO DETERMINE THE RELATIVE NUMBERS OF UNPRODUCTIVE FEMALES*

Date mated (1946)	Average temperature during oviposition		Number of females					
			Dinuba stock			Southern California stock		
	Daily mean	Maxi- mum	Observed	Not laying	Laying only nonviable eggs	Observed	Not laying	Laying only nonviable eggs
Apr. 5	62.8	75.1	14	1	0	12	0	1
Apr. 22	68.3	85.2	15	1	0	15	0	1
June 3	69.0	83.8	15	2	0	15	0	0
June 14	74.6	89.9	15	4	3	15	0	1
June 23	75.6	89.8	14	1	3	15	0	0
July 6	78.9	94.3	15	1	1	15	0	0
July 23	80.4	93.6	15	1	3	15	0	0
Aug. 1	82.5	99.4	15	4	3	15	0	3
Aug. 13	79.3	95.3	15	2	2	15	0	0
Totals	133	17	15	132	0	6

* The pooled chi-square for stock differences in reproductive status of females—productive or unproductive, including deficiencies of both types—is significant; $\chi^2 = 18.88$, $P < 0.01$.

nomenon was not the sole factor responsible for stock differences in oviposition. There appeared to be some inherent difference in laying capacity, as attested by the average calculated for the fertile females, namely 84.2 eggs per female for Dinuba females and 116.4 eggs per female for the southern California females.

The temperature data of table 7 suggest that hot weather retards reproduction by increasing the number of unproductive females. That such is the case, however, is not apparent in the material presented earlier (tables 5 and 6).

VIABILITY OF THE EGGS

In the routine handling of eggs affixed to wax paper it was noticed that there was always a small percentage of eggs which did not hatch and, furthermore, that the percentage appeared to increase during hot weather. In view of the second observation, the percentages of eggs hatched were determined for relatively large batches of eggs incubated under varying conditions of summer temperature and humidity. As a check on the procedures involving wax paper, percentages of eggs hatched were also determined for eggs laid on peach leaves.

As a preliminary step in establishing procedures, information was desired as to the advisability of making up samples without regard to the ages of

the ovipositing moths. Therefore data were assembled from available records of hatching and compiled as shown in table 8. The ages of the female moths are given in class intervals of 5 days; and the number of eggs deposited, together with the per cent hatched, are totaled for all of the females within each 5-day interval of age. The eggs having the lowest viability were deposited by females of the middle age class (11 to 15 days). This was also the age class in which the largest number of females ceased to oviposit. In other words, the females which laid for shorter periods contributed a larger proportion of nonviable eggs than those which laid for longer periods. The longer-lived

TABLE 8
HATCHABILITY OF EGGS CLASSIFIED ACCORDING TO AGE OF FEMALE MOTHS, 1946

Stock	Number of females	Age of females in days				
		2-5	6-10	11-15	16-20	21-25
		Number of eggs laid Per cent of eggs hatched				
Dinuba...	169	2,319 82.4	4,432 75.2	3,062 69.8	784 80.6	153 85.6
Southern California...	75	4,339 94.8	3,789 90.7	1,724 72.2	385 93.1	40 97.5

females continued to oviposit for periods ranging up to 25 days. The eggs deposited by these older individuals proved to be as viable as those deposited at the beginning of oviposition. The data imply that the variations in the hatching as revealed in table 8 are related to differences in vitality or physiological condition of the moths, as evidenced by the duration of oviposition and, possibly, by the size of the brood. In view of the drop in oviposition and in viability of eggs with females 11 to 15 days old, the eggs used in experiments to be described were taken only from cages containing moths 2 to 10 days of age.

In one series of tests, samples of eggs were incubated at various intervals during the change from cool to hot weather, between April 25 and August 10, 1946, in order to compare the percentages of eggs hatched on wax paper during incubation periods having different average temperatures. In each test, three samples of eggs, one for each of three stocks, were incubated under uncontrolled conditions in the insectary. Table 9 presents the results obtained from the hatching of 71,047 eggs. The tests are arranged in the table according to ascending order of average maximum temperatures during incubation.

The experiment summarized in table 9 showed that the viability of the eggs deposited on wax paper declined during the weather changes between spring and midsummer, and that the eggs deposited by three stocks of moths were similarly affected. The hatching of the eggs of the Dinuba stock declined 20.3 per cent, from a high of 94.8 per cent on June 11 to a low of 74.5 per cent on August 8. The decline in hatching for eggs of the southern California stock was 21.6 per cent, and for those of the Parlier I stock, 27.2 per cent. An inverse

relation between percentages of eggs hatched and the average temperatures during incubation is evident in the data. In order to find which of the three sets of average temperatures gave the closest correlation with per cent of eggs hatched, coefficients of correlation (r) were calculated by using one set of temperature values at a time. The largest coefficients were obtained when the

TABLE 9
PERCENTAGES OF EGGS HATCHED ON WAX PAPER WHEN INCUBATED AT
VARIOUS TEMPERATURES

Date laid (1946)	Temperatures of insectary during incubation			Dinuba stock		Southern California stock		Parlier I stock	
	Average maximum	Greatest maximum	Average daily mean	Number of eggs observed	Per cent hatched	Number of eggs observed	Per cent hatched	Number of eggs observed	Per cent hatched
Apr. 25	81.6	87	66.1	1,863	90.8	817	90.7	605	81.2
Apr. 23	85.8	94	68.9	1,643	93.1	989	88.8	524	90.5
June 11	86.8	89	70.7	961	94.8	2,785	94.3	1,633	94.8
June 12	87.0	90	72.0	1,052	91.3	2,075	91.3	1,472	87.9
Apr. 20	87.3	94	69.5	583	91.1	742	85.9	473	80.1
Apr. 21	88.0	94	70.1	712	91.1	1,001	89.0	383	85.1
Apr. 22	88.2	94	70.3	1,894	90.8	1,440	88.1	931	85.8
June 13	88.2	94	72.8	1,554	92.6	3,019	90.2	618	89.6
July 12	92.4	97	77.4	1,945	93.0	3,334	87.8	1,491	86.7
Aug. 12	93.8	96	77.4	3,115	79.7	4,347	81.0	2,186	75.9
July 16	97.3	100	83.1	2,764	87.7	3,761	82.0	3,692	84.5
Aug. 10	97.3	101	81.8	3,012	87.0	2,678	84.7	2,015	80.0
Aug. 8	99.7	101	84.2	1,774	74.5	3,771	72.7	1,393	67.6
Total or weighted mean.	22,872	88.3	30,759	85.9	17,416	83.8

Stocks		Degrees of freedom	Total chi-square
Dinuba-Southern California		12	121.00*
Southern California-Parlier I		12	108.87*
Dinuba-Parlier I		12	223.72*

* $P < 0.01$.

average maximum temperatures were used as one set of variates. The average daily mean and the greatest maximum temperatures gave slightly lower coefficients (table 10).

The eggs of the three stocks appeared to differ in viability. The chi-square test was applied to the data of table 9 by computing a sum of chi-squares for the samples by considering two stocks (columns) at a time (see table 9).

A second experiment, similar to the first in method, was made to compare the percentages of eggs hatched when incubated on wax paper with the percentages hatched when incubated on peach leaves. The peach leaves were handled as bouquets of twigs having their cut ends plugged into vials of water. While the eggs were incubating, the twigs were kept in the insectary, shielded from the sun but not from air currents. The eggs on wax paper were also incubated under uncontrolled conditions in the insectary. Those used in each test were deposited on both leaves and wax paper by the same batch of moths,

that is, the twigs and paper were placed in the same cage. Different batches of moths, southern California stock, supplied the eggs for tests set up on different dates.

In table 11 the results of fifteen tests are arranged in ascending order of average maximum temperatures during incubation. In view of possible differences in surface moisture and aëration, separate counts were made for eggs incubated on the upper and lower surfaces of the leaves. There are no stomata in the upper epidermis of peach leaves, whereas there are approximately 22,500 stomata per square centimeter in the lower epidermis (Miller, 1938).

TABLE 10
COEFFICIENTS OF CORRELATION BETWEEN PER CENT OF EGGS HATCHED
AND TEMPERATURES DURING INCUBATION; DATA FROM TABLE 9

Temperatures during incubation	Coefficients of correlation		
	Dinuba stock	Southern California stock	Parlier I stock
Average maximum.....	-0.72	-0.82	-0.56
Average daily mean	-0.71	-0.78	-0.54
Greatest maximum.....	-0.59	-0.71	-0.53

The ranges of variation in percentages of eggs hatched during this experiment were noticeably less than those observed in the previous experiment, but the range of average temperatures during incubation was also smaller. The range of difference in viability was 9.7 per cent for eggs incubated on the lower leaf surfaces. For eggs incubated on the upper leaf surfaces the range was 11.8 per cent, and for eggs incubated on wax paper it was 11.2 per cent. The variations introduced into the experiment through the use of eggs laid by different batches of moths almost obscured the variations due to temperature differences. For example, the eggs incubated at the intermediate temperatures hatched in greater numbers than those incubated at the lower temperatures. In general, the percentages hatched in those tests made at the highest temperatures were but slightly below the weighted means for the experiment.

On the basis of the results obtained in the second experiment it is concluded that the viability of the eggs was not appreciably affected by midsummer weather conditions when they were incubated on living peach leaves.

The results further indicate that the viability was greater for eggs incubated on peach leaves than for those incubated on wax paper, and also that viability was slightly greater on the lower than on the upper surface of the peach leaves.

The mortality of the eggs incubated on wax paper in the second experiment was somewhat lower than in the first experiment or with previous observations. In the first experiment, the eggs were deposited in cages containing only small pads of wet cotton, one pad per cage. In the second experiment, they were laid in cages containing bouquets of transpiring peach twigs in addition to the wet pads. The amount of foliage was large in relation to the volume of the cages. The higher mortality shown in the first instance may

mean that the eggs were especially susceptible to dry atmospheric conditions during the first few hours after being deposited.

In the first of the hatching experiments moderately good inverse correlations were obtained between percentages of eggs hatched on wax paper and the average maximum temperatures during incubation. This does not mean that the variations in hatching were due exclusively to temperature changes;

TABLE 11
PERCENTAGES OF EGGS HATCHED ON PEACH LEAVES AND ON WAX PAPER WHEN INCUBATED
AT VARIOUS TEMPERATURES

Date laid (1946)	Temperatures of insectary during incubation			Lower surface of leaves		Upper surface of leaves		Wax paper	
	Average maxi- mum	Greatest maxi- mum	Average daily mean	Number of eggs observed	Per cent hatched	Number of eggs observed	Per cent hatched	Number of eggs observed	Per cent hatched
June 18..	91.6	101	76.2	1,181	92.5	211	93.4	622	88.8
July 22..	92.5	97	82.0	2,648	93.8	1,886	92.6	885	90.2
July 23....	92.5	97	81.8	2,059	94.9	1,106	92.5	913	90.4
July 12.....	92.6	97	77.5	1,679	94.8	974	94.1	1,059	89.3
July 11....	93.0	99	78.2	1,896	94.8	1,388	94.7	1,384	89.8
July 13....	93.6	100	77.6	1,871	94.0	798	95.4	634	90.4
July 28.....	93.8	101	78.3	2,361	96.1	924	94.7	1,098	93.1
June 17.....	94.2	101	77.6	1,034	90.6	459	85.5	442	85.8
Aug. 13.....	94.4	98	77.5	1,790	94.1	600	95.2	2,035	90.3
Aug. 14.....	94.8	98	77.9	2,317	93.5	959	94.6	2,254	89.0
Aug. 15....	94.8	98	78.3	1,381	95.5	897	95.1	1,822	92.2
June 16....	95.4	101	77.9	1,229	95.5	360	93.6	440	84.1
Aug. 5.....	96.4	101	80.2	1,448	86.4	1,308	83.6	1,170	81.9
Aug. 6.....	97.5	101	81.3	1,520	92.8	1,215	90.0	1,199	87.9
July 29....	98.4	104	81.0	2,737	91.5	1,592	87.9	666	92.2
Total or weighted mean.	27,151	93.8	14,677	92.1	16,623	89.3

Surface		Degrees of freedom	Total chi-square
Lower leaf-upper leaf.		14	50.51*
Upper leaf-wax paper.		14	132.20*
Lower leaf-wax paper.		14	274.86*

* $P < 0.01$.

for, in general, increases in temperature were usually accompanied by decreases in relative humidity, and vice versa. Furthermore, the characteristics of the wax paper may have altered in the higher temperature ranges. Some information about the effect of differences in humidity on the viability of eggs adhering to wax paper was obtained from the following experiment. A large number of ovipositing moths were placed in a cage containing both wax paper and peach twigs. On the following morning the eggs on the twigs and paper were removed from the cages and immediately prepared for incubation. The twigs, already plugged into water-filled vials, were placed upright on a table in an open part of the insectary. The eggs affixed to the wax paper were divided into two portions and each portion was put into a shallow receptacle. One portion was put aside on a rack to incubate under uncontrolled conditions. The other portion was placed in a humidifier. The latter consisted of a battery

jar partly filled with wet sand and a cover of muslin. While in use, the sand in the humidifier was kept wet to the point of saturation. Batches of eggs deposited by the same moths were treated in this manner for 3 days in succession (July 11, 12, and 13). The three batches of eggs were combined and treated as one lot (lot A, table 12). The procedure was repeated for three more batches (lot B) at a later time (August 13, 14, and 15).

The outcome of the experiment is shown in table 12. The percentages of eggs hatched on peach leaves were greater than the percentages hatched on

TABLE 12
PERCENTAGES OF EGGS HATCHED WHEN INCUBATED ON WAX PAPER UNDER CONDITIONS OF HIGH AND LOW HUMIDITY AND ON PEACH LEAVES, 1946

Lot	Temperatures of insectary during incubation			Average per cent relative humidity of insectary		Lower leaf surface		Wax paper in moist chamber		Wax paper in dry tray	
	Average maximum	Greatest	Average daily mean	Maximum	Minimum	Number of eggs observed	Percent hatched	Number of eggs observed	Percent hatched	Number of eggs observed	Percent hatched
A ..	95.1	100	79.6	87	27	5,446	94.5	7,969	90.4	3,069	89.7
B ..	94.3	96	77.8	88	27	5,488	94.2	7,658	92.7	6,111	90.4

Computed chi-square
(*d.f.* = 1)

Lower leaf surface and wax paper (humidified)		Wax paper (humidified) and wax paper (dry tray)	
A	75.57*	A.....	0.93
B.....	11.78*	B.....	23.12*

* $P < 0.01$.

either the dry or humidified wax paper. Also, in both trials, the percentages hatched on humidified wax paper were greater than the percentages hatched on wax paper under insectary conditions, although only one trial yielded a difference great enough to be significant. Increasing the moisture content of air surrounding the eggs on wax paper, from midsummer conditions to near saturation, resulted in some increase in the number of eggs hatched. Yet neither hatch obtained in the moist chamber equalled that obtained from eggs on the leaves. Probably surface temperature regulation by the leaves and surface ventilation are additional factors affecting the viability of eggs on leaves. The eggs on the leaves of the cut twigs probably developed at temperatures several degrees lower than the air temperatures in the interior of the insectary. According to Miller (1938), the temperature of leaves in direct sunlight, as measured on the upper leaf surfaces of several crop plants, are apt to fluctuate rapidly above and below the temperature of the air. In diffuse sunlight, however, the temperature of attached turgid leaves averages from 0.1° to 3.0° C below air temperatures (Miller and Saunders, 1923). It is further known that air currents tend to reduce leaf temperatures (Smith, 1909).

Eggs on wax paper were also less viable when incubated under uncontrolled conditions than when incubated with the egg side in contact with a layer of green peaches packed in muslin-covered wooden trays. The results of two replicate counts are given in table 13.

TABLE 13
PERCENTAGES OF EGGS HATCHED ON WAX PAPER IN DRY TRAYS AND IN TRAYS CONTAINING GREEN PEACHES

Date laid (1946)	Temperatures in insectary during incubation, ° F			Replicate	Dry trays		Over peaches		Chi- square (d. f. = 1)
	Average daily mean	Average maxi- mum	Greatest maxi- mum		Number of eggs observed	Per cent hatched	Number of eggs observed	Per cent hatched	
July 16.. . . .	81.9	97.3	100	A	1,760	81.1	2,041	91.3	82.35*
				B	2,001	82.7	2,237	92.6	97.41*

* $P < 0.01$.

DISCUSSION

The question of the relation between the local summer climate and Oriental fruit moth propagation arose during 1944 and 1945, when the numbers of eggs deposited and the percentages of eggs hatched on wax paper were observed to decline considerably during hot weather. Moreover, marked increases in oviposition were noted when, during hot weather, the maximum temperatures (ranging from 92° to 107° F) were reduced approximately 10 degrees by the construction of evaporative cooling systems over the cages. Other observers have also commented on the adverse effect of high temperatures on oviposition. Garman (1930) found that the Oriental fruit moths in cages often suffer and may die without laying at temperatures above 90°. Snapp and Swingle (1929) observed that temperatures above 100° greatly reduce the number of eggs laid and that maximum laying occurs on days having a range of 70° to 95°. The optimum range for oviposition is given as 70° to 90° by Peterson and Haeussler (1930).

Averages of the number of eggs laid during 1944 and 1945 did not exceed 35 eggs per female for moths of the Dinuba stock. The larvae from which they were derived were reared on apples exclusively during 1944. During 1945, some of the larvae were reared on peaches but the majority were reared on apples, commercial Pippins (winter stored) in the spring months and small green fruit after thinning time. The cocooned larvae were not differentiated with respect to type of food or duration of feeding. They were taken from feeding trays as long as the fruit was not badly soured or contaminated with molds. All of the cocoons were put into a common emergence cage.

The crowding of larvae within the fruit also may have affected the size and fecundity of the moths. The wax papers to which the eggs adhered were cut and arranged on the apples or peaches so that about ten larvae would enter each of the fruits. Dustan's (1935) experiments indicate that the rearing of ten larvae per fruit impairs the fecundity of the adult females. When reared as two larvae per apple, adult females averaged 39 eggs each; but when reared as ten larvae per apple, the adult females averaged only 14.5 eggs each.

In the tests conducted during 1946, moths of the same stock deposited more eggs than before and, furthermore, oviposition did not break down in mid-summer. The increased vitality during the third year was probably due to a change in the type of food used for rearing the larvae and to the selection of larvae for size and feeding time. Green peaches were used exclusively in 1946. It is more difficult to use peaches on a large scale because they are apt to decompose badly when infested with immature larvae, especially in hot weather. A standard procedure was adopted in which only the first larvae to emerge, the best in color and size, were withdrawn from the feeding trays. Those appearing in trays having partly rotten or molded fruit were discarded.

It now appears that the earlier results were due to inadequacies of the methods rather than to severe summer climate. Propagation of the Oriental fruit moth was maintained at a high level throughout the summer when the moths were obtained only from vigorous larvae reared on green peaches and when peach foliage instead of wax paper was used as an incubating medium for the eggs.

The most prolific of the insectary moths (southern California stock) laid 114.1 eggs as an average for 884 females. The highest average obtained for any one test was 143.0 eggs per female for 100 females. The average for the season is considerably greater than hitherto obtained for Oriental fruit moths by other observers, and yet it is believed to be a low estimate of the maximum production for the species under optimum outdoor conditions. High averages for single tests have been obtained in two other insectaries. In one test made by Peterson and Haeussler (1930) at Ironton, New Jersey, 100 females averaged 148 eggs each. At Wooster, Ohio, Neiswander (1936) reported an average of 129.0 eggs per female from three cages each containing 10 females. Steiner and Yetter (Yetter and Steiner, 1931; Steiner and Yetter, 1933) estimated Oriental fruit moth reproduction by determining the egg content of 750 dissected females. The average number of eggs in preovipositional females was 141 eggs in 1931 and approximately 200 in 1932. The maximum number of eggs found in any one female was 362. They found no evidence that additional immature eggs form after the first day of adult life. The higher counts during 1932 were thought to be one of the causes for a heavier orchard infestation during that year.

The work leading to the differentiation of several stocks of moths was done as a routine check on the original stock of insectary moths to determine whether there were genetic differences in responsiveness to hot-weather conditions. The fact that two stocks differed in oviposition and three stocks in viability of eggs is of problematic significance. The differences may have originated as genetic segregations in consequence of laboratory procedures in breeding. On the other hand, it may mean that the populations from which the samples were taken differ in reproductive potential and that they have not yet intermingled.

SUMMARY

Oriental fruit moths were found to mate successfully as early as the day of emergence. Individuals of both sexes appeared to retain the ability to reproduce after remaining unmated for relatively long periods of time. One male usually mated successfully with only one female during one 12-hour

period of association, although in the same period additional females present were occasionally induced to lay small numbers of nonviable eggs. Females inseminated by one or more males during one 12-hour period of association oviposited normally thereafter in the absence of males.

Comparative ovipositional tests were made for two stocks of moths, one obtained from Orange County, in southern California, and the other from Dinuba, in central California. When reared and tested under the same conditions, females of the southern California stock deposited, as an average, about twice as many eggs as the females of the Dinuba stock. Oviposition tests were also made for two additional stocks, Parlier I and Parlier II, but they were not clearly differentiated from each other or from the Dinuba stock.

The average numbers of eggs deposited by females during 7-day laying periods were compared for groups of females laying at different times between April 18 and September 9, 1946. The data show that two of the three stocks tested (southern California and Dinuba) gave the highest production averages during the hottest part of the summer, July 10 to August 8. Moths of the third stock (Parlier I) oviposited about as well in hot weather as in cool weather.

When eggs affixed to wax paper were incubated under uncontrolled conditions, the percentages hatched were found to vary inversely with the average temperatures during incubation. For eggs deposited by moths of three stocks, the highest percentages hatched in individual samples were approximately 95 per cent, whereas the percentages hatched in samples incubated at the highest average summer temperatures ranged from 74.5 per cent for eggs of the Dinuba stock to 67.6 per cent for eggs of the Parlier I stock.

There were significant differences in the viability of eggs laid by moths of the three strains.

During hot weather, the percentages of eggs hatched after being incubated on living peach leaves were greater than the percentages obtained for eggs incubated on wax paper under laboratory conditions. The percentages ranged from 96.1 to 83.6 for samples of eggs incubated on peach leaves as compared with a range of 93.1 to 81.9 for samples of eggs incubated on wax paper. The viability of eggs incubated on wax paper was slightly increased by increasing the atmospheric moisture. The viability of eggs deposited on the lower surfaces of peach leaves was slightly greater than the viability of those incubated on the upper surfaces.

The viability of eggs incubated on peach leaves was not greatly reduced by hot-weather conditions: more than 90 per cent of the eggs hatched in 26 out of 30 samples (36,430 out of 41,237 eggs) which were incubated during the period June 16 to August 15, 1946.

Evidence was obtained to show that differences in atmospheric moisture account only in part for differences between the percentages of eggs hatched on dry wax paper and on transpiring peach leaves.

From these experiments it does not appear that propagation of the Oriental fruit moth is seriously impaired by the high temperature and low humidity factors in the summer climate of central California.

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CONTENTS

	Page
APHID TRANSMISSION OF CAULIFLOWER-MOSAIC VIRUS, by Henry H. P. Severin and C. M. Tompkins	389
Summary	389
Introduction	390
Materials and methods	390
Symptomatology	391
Tests with plant bugs and leafhoppers	392
Aphid transmission of virus	392
Susceptibility of varieties of cauliflower	394
Comparative efficiency of mechanical inoculation and aphid transmission of virus	394
Retention of virus	397
Recovery of virus by aphids from an inoculated plant before symptoms develop	400
Mechanical inoculation with virus extract from crushed aphids	401
Literature cited	402
THE MOST IMPORTANT SPECIES OF APHIDS ATTACK- ING CRUCIFEROUS CROPS IN CALIFORNIA, by E. O. Essig	407
The cabbage aphid	407
The turnip aphid	412
The green peach aphid	414
Literature consulted	420

APHID TRANSMISSION OF CAULIFLOWER- MOSAIC VIRUS¹

HENRY H. P. SEVERIN² and C. M. TOMPKINS³

SUMMARY

ELEVEN SPECIES OF APHIDS that do not multiply on cauliflower plants in nature were demonstrated to be vectors of the cauliflower-mosaic virus, five of them being more efficient vectors than three aphid species that do breed on cauliflower.

Of three aphid species that breed on cauliflower under natural conditions, the cabbage aphid, *Brevicoryne brassicae* (Linnaeus), was a more efficient vector of the virus (in single-insect tests) than the turnip or false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis), and the green peach aphid, *Myzus persicae* (Sulzer).

Natural infectivity of the cabbage aphid was demonstrated.

None of twenty-one varieties of cauliflower experimentally infected with the virus by the cabbage and green peach aphids was resistant to the disease.

Mechanical inoculation was more efficient than transmission by the cabbage, turnip, or green peach aphid.

In tests with hourly transfers, most transmissions occurred within 2 hours after the aphids had fed on a mosaic-infected plant.

Aphids acquired the virus in 15 to 25 minutes on a diseased plant (5 to 10 minutes actual feeding time) and transmitted it in feeding periods as short as 5 or 10 minutes. In tests with short feeding periods, most of the transmissions occurred during the first 10 minutes after transfer from the infected plant, but an occasional aphid transmitted the disease during the second or third 10-minute period.

The cabbage and green peach aphids recovered the virus from inoculated cauliflower plants many days before the first symptom appeared.

Mechanical inoculation of healthy cauliflower seedlings and annual stock plants with the centrifuged virus extract from crushed, infective cabbage aphids produced 18 per cent infection.

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INTRODUCTION

Cauliflower is naturally infected with cauliflower mosaic in California (Tompkins, 1934a)⁴ and in Oregon (Tompkins, 1937). Cauliflower plants have been experimentally infected with cauliflower-mosaic virus by mechanical inoculation (Tompkins, 1934a). The host range and properties of the virus have been reported (Tompkins, 1937).

Cauliflower-mosaic virus is transmitted by the cabbage aphid, *Brevicoryne brassicae* Linnaeus, and the green peach aphid, *Myzus persicae* (Sulzer) (Tompkins, 1934a, 1937). Keicola (1945) demonstrated that *M. persicae* and *B. brassicae*, when colonized on cauliflower seedlings infected with both cabbage black ringspot (cabbage ringspot) and cauliflower mosaic, transmitted both viruses; whereas *M. ornatus*, when similarly colonized, transmitted only the cauliflower-mosaic virus.

An investigation was undertaken to obtain further information on aphid transmission of the cauliflower-mosaic virus. Tests were made both with species that do not breed on cauliflower under natural conditions and with species that do. Other aspects investigated include the efficiency in the transmission of the virus by single aphids and by varying numbers of aphids; susceptibility of cauliflower varieties to the disease; comparative efficiency of mechanical inoculation and aphid transmission; the natural infectivity of the aphids; retention of the virus by aphids; and whether infections could be obtained with the virus extract from crushed, infective aphids.

In a companion paper, Essig (1948) discusses the characters, distribution, and food plants of aphid species which occur on cauliflower plants under natural conditions in California.

Leafhoppers transmit two other viruses to cauliflower: cauliflower plants experimentally infected with the curly-top virus by means of the beet leafhopper, *Eutettix tenellus* (Baker), developed symptoms, though noninfective leafhoppers failed to recover the virus (Severin, 1929); and California aster yellows has been recovered from naturally infected cauliflower and transferred to healthy aster and celery plants by previously noninfective aster leafhoppers, *Macrostelus divisus* (Uhler) (Severin and Frazier, 1945). Hence, tests of cauliflower-mosaic virus transmission by species of leafhoppers were included in this investigation. Transmission by a plant bug naturally occurring on cauliflower was also tested.

MATERIALS AND METHODS

The virus was obtained from severely stunted plants of the February variety of cauliflower at Alvarado. It was maintained by repeated mechanical inoculation and aphid transmission of the virus to healthy February cauliflower and Fiery Blood Red annual stock plants. The carborundum method (Rawlins and Tompkins, 1936) was used in mechanical inoculations.

Colonies of noninfective aphid species were started by transferring mature, wingless aphids from cauliflower, cabbage, or stock to Esmarch dishes with the bottoms covered with moist filter paper. The young aphids were then placed on healthy cauliflower or annual stock plants grown from seeds. The

⁴ See "Literature Cited" for citations, referred to in the text by author and date.

method of transferring aphids has been published in a previous paper (Severin and Freitag, 1938). The preparation of the virus extract from crushed, infective aphids and by centrifugation was similar to the method described for the beet leafhopper (Severin and Freitag, 1933).

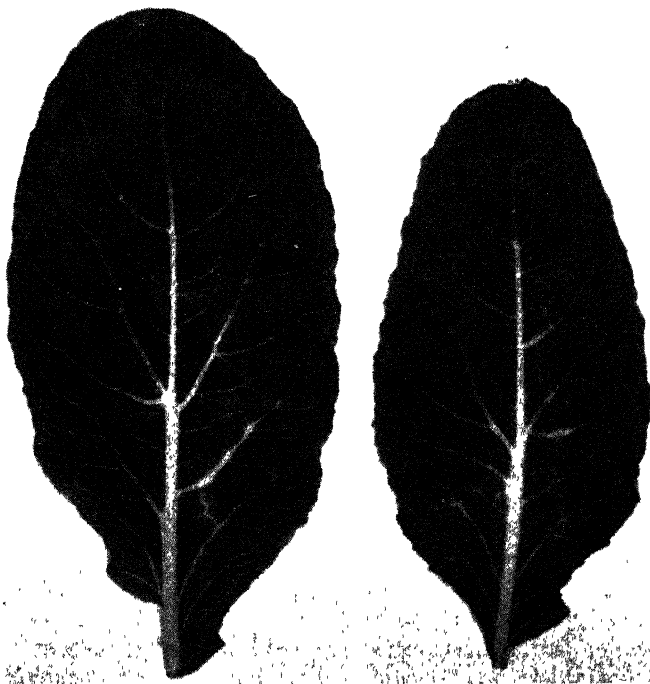


Fig. 1. Local symptoms of cauliflower mosaic on leaves of cauliflower seedling, on which the cabbage aphid had fed, showing chlorotic areas around mouth-part punctures.

SYMPTOMATOLOGY

A general description of the symptoms of cauliflower mosaic on several host plants including annual stock was given in a previous paper (Tompkins, 1937) : clearing of the veins was the predominant symptom; stunting was not observed. The average incubation period of the disease on Fiery Blood Red annual stock (*Mathiola incana* var. *annua*) was 70 days.

In cauliflower, mosaic is characterized by stunting of the plant, dwarfing of the terminal head or curd, and the following leaf symptoms: vein clearing (plate 1, *B*) ; curvature of the midrib (plate 1, *D*) ; necrotic lesions (plate 1, *E*, *F*) and distortion of the leaf (plate 1, *G*).

Feeding by the cabbage aphid causes symptoms on cauliflower, aside from those caused by the virus. The leaves of cauliflower seedlings on which either noninfective or infective cabbage aphids had fed showed circular chlorotic areas around the mouth-part punctures (fig. 1). The newly developing leaves on which no aphids had fed failed to show this symptom; hence the effect is local and not systemic. Other species of aphids do not cause this symptom.

Since annual stock has been experimentally infected with five crucifer viruses (Tompkins, 1934b, 1937, 1938, 1939; Tompkins, Gardner, and Thomas, 1938), a detailed description of the successive foliage symptoms of cauliflower mosaic on stock is given to distinguish this virus disease from others, especially from mild and severe mosaics of stock plants, described in a previous paper (Tompkins, 1939). The first symptom that appears on the youngest leaves of Fiery Blood Red annual stock is a clearing of the veins and veinlets (plate 2, *C*), followed by the development of numerous chlorotic, circular areas (plate 2, *D*), producing a mottled effect (plate 2, *E*). The veins may protrude on the lower surface of the leaf (plate 1, *F*). A shortening of the internodes occurs, and secondary shoots develop with dwarfed leaves cupped inward along the midrib (plate 2, *A*).

TESTS WITH PLANT BUGS AND LEAFHOPPERS

Tests were made to determine whether plant bugs and leafhoppers occurring in cauliflower fields could transmit the virus of cauliflower mosaic. The harlequin cabbage bug, *Murgantia histrionica* (Hahn), was abundant on cauliflower plants in San Pablo. Lots of 2 to 5 adults were transferred, alternating daily from mosaic to healthy cauliflower plants. No infections occurred with 30 cauliflower plants tested. No infections were obtained with the beet leafhopper, short-winged and long-winged aster leafhoppers, *Macrostelus divinus* (Uhler); the geminate leafhopper, *Colladonus geminatus* (Van Duzee); or the mountain leafhopper, *C. montanus* (Van Duzee).

APHID TRANSMISSION OF VIRUS

By Vectors That Do Not Breed on Cauliflower. Aphids that have not been found to breed on cauliflower plants under natural conditions were tested for transmission of the virus of cauliflower mosaic. The aphids were transferred singly, directly from diseased plants to healthy cauliflower seedlings, one aphid to a plant, and were kept on the healthy plants until they died. Most of the species tested proved capable of transmitting the virus (table 1). There was a considerable variation in the percentages of virus transmission by the different species. The most important vectors were the pea aphid, *Macrosiphum pisi* (Kaltenbach); celery aphid, *Aphis apii* Theobald;⁵ lily aphid, *Myzus circumflexus* (Buckton); and yellow willow aphid, *Cavariella capreae* (Fabricius). Others that transmitted the virus were the celery leaf aphid, *Aphis apigraveolens* Essig; rusty-banded aphid, *Aphis ferruginea-striata* Essig; cotton or melon aphid, *Aphis gossypii* Glover; erigeron root aphid, *Aphis middletonii* (Thomas); foxglove aphid, *Myzus solani* (Kaltenbach); and honeysuckle aphid, *Rhopalosiphum conii* (Davidson). The bean or dock aphid, *Aphis rumicis* Linnaeus, failed to transmit the virus. It may be possible that species of winged aphids play an important role in the dissemination of the virus.

By Single Aphids Breeding on Cauliflower. Three aphid species that breed on cauliflower under natural conditions were tested for efficiency as vectors of the virus; these were the cabbage aphid, *Brevicoryne brassicae* (Linnaeus);

⁵ According to E. O. Essig (personal interview), *Aphis apii* Theobald may be identical with *A. helianthi* Monell.

turnip or false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis); and green peach aphid, *Myzus persicae* (Sulzer). Tests were made with wingless and winged aphids, reared to maturity on mosaic-infected cauliflower and then transferred, each to a separate healthy cauliflower plant. Table 2 compares the number of infections obtained with single winged and wingless aphids. Of the three species of aphids tested singly, the cabbage aphid is the most efficient vector of the virus.

TABLE 1
TRANSMISSION OF CAULIFLOWER-MOSAIC VIRUS BY
SINGLE APHID SPECIES

Species of aphids	Cauliflower		Per cent infected
	Inoculated	Infected	
Celery leaf aphid, <i>Aphis apigraveolens</i>	35	2	6
Celery aphid, <i>Aphis apii</i> *.....	35	25	71
Rusty-banded aphid, <i>Aphis ferruginea-striata</i>	85	4	5
Cotton or melon aphid, <i>Aphis gossypii</i>	50	2	4
Erigeron root aphid, <i>Aphis middletonii</i>	75	1	1
Bean or dock aphid, <i>Aphis rumicis</i>	80	0	0
Yellow willow aphid, <i>Cavariella capreae</i>	35	20	57
Pea aphid, <i>Macrosiphum pisi</i>	35	30	86
Lily aphid, <i>Myzus circumflexus</i>	35	23	66
Foxglove aphid, <i>Myzus convolvuli</i>	35	10	29
Honeysuckle aphid, <i>Rhopalosiphum conii</i>	50	1	2

See footnote 5, page 392.

TABLE 2
TRANSMISSION OF VIRUS BY SINGLE APHIDS THAT BREED ON
CAULIFLOWER UNDER NATURAL CONDITIONS

Species of aphids	Results with wingless mature aphids			Results with winged aphids		
	Cauliflower			Cauliflower		
	Inoculated	Infected	Per cent infected	Inoculated	Infected	Per cent infected
Cabbage aphid, <i>Brevicoryne brassicae</i>	150	28	15	100	22	22
Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i> ...	200	8	4	100	5	5
Green peach aphid, <i>Myzus persicae</i>	100	11	11	150	4	3

From Naturally Infected Cauliflower and Brussels Sprouts. Whenever high populations of the cabbage aphid were found on mosaic-infected cauliflower plants, lots of 20 aphids were transferred to healthy cauliflower and annual stock plants. Table 3 shows the counties and districts from which the source of virus was obtained. The cabbage aphid transmitted the virus to 67 per cent of the cauliflower and to 43 per cent of the annual stock plants. Virus from the Richmond and Colma districts was transmitted to cauliflower but not to stock.

The virus was also transmitted by the cabbage aphid from naturally infected brussels sprouts obtained at El Granada to healthy cauliflower seedlings.

From Experimentally Infected Cauliflower to Healthy Cauliflower and Stock Plants. Lots of 20 cabbage, turnip, and green peach aphids were used in determining the transmission of the virus from mosaic-infected cauliflower to healthy cauliflower seedlings and annual stock plants. Table 4 shows the number and percentages of cauliflower and annual stock plants infected with the virus by means of three species of aphids.

TABLE 3
TRANSMISSION OF CAULIFLOWER-MOSAIC VIRUS FROM NATURALLY
INFECTED TO HEALTHY CAULIFLOWER AND STOCKS BY LOTS OF
20 CABBAGE APHIDS, *BREVICORYNE BRASSICAE*

County and district	Cauliflower		Stocks	
	Inoculated	Infected	Inoculated	Infected
Alameda county:				
Alameda.....	6	3	6	2
Contra Costa County:				
Richmond.....	6	2	6	0
San Pablo.....	6	6	6	4
San Francisco County:				
San Francisco.....	6	3	6	4
San Mateo County:				
Colma.....	6	6	6	4
El Granada.....	6	4	6	0
Monterey County:				
Salinas.....	6	4	6	4
Total.....	42	28	42	18
Percentage.....	67	43

Annual stock plants infected with the virus by the three species of aphids did not show breaking in the color of the petals, as previously reported (Tompkins, 1937). Breaking in the flowers of annual stock is caused by two other viruses (Tompkins, 1934*b*, 1939*a*).

SUSCEPTIBILITY OF VARIETIES OF CAULIFLOWER

Plants of twenty-one varieties of cauliflower were inoculated with the virus to ascertain whether any variety was resistant to the disease. Lots of 20 infective cabbage aphids and green peach aphids were transferred from diseased to healthy cauliflowers, one lot to a plant. Table 5 shows that 78 per cent of the cauliflower seedlings were infected by means of the cabbage aphid and 58 per cent by the green peach aphid. Again, the cabbage aphid was a more efficient vector of the virus than the green peach aphid. All varieties of cauliflower were highly susceptible to cauliflower mosaic.

COMPARATIVE EFFICIENCY OF MECHANICAL INOCULATION AND APHID TRANSMISSION OF VIRUS

The transmission of virus from experimentally infected to healthy cauliflower plants by mechanical inoculation was compared with transmissions by three species of aphids. A summary of virus transmission by aphids (single

TABLE 4
TRANSMISSION OF CAULIFLOWER-MOSAIC VIRUS FROM INFECTED
CAULIFLOWER TO HEALTHY CAULIFLOWER SEEDLINGS AND
ANNUAL STOCKS BY THREE APHID SPECIES

Aphid species and test no.	Cauliflower		Stocks	
	Inoculated	Infected	Inoculated	Infected
<i>Cabbage aphid, Brevicoryne brassicae:</i>				
Test 1	10	0	10	9
Test 2	5	5	5	5
Test 3	5	5	5	3
Test 4	5	5	5	3
Test 5	5	4	5	5
Test 6	5	3	5	2
Test 7	5	3	5	0
Total	40	25	40	27
Percentage	63	69
<i>Turnip aphid, Rhopalosiphum pseudobrassicae:</i>				
Test 1	5	3	5	3
Test 2	5	3	5	3
Test 3	5	3	5	3
Test 4	5	3	5	1
Test 5	5	2	5	3
Test 6	5	0	5	3
Total	30	14	30	16
Percentage	47	53
<i>Green peach aphid, Myzus persicae:</i>				
Test 1	10	10	10	9
Test 2	5	5	5	5
Test 3	5	5	5	2
Test 4	5	4	5	5
Test 5	5	4	5	3
Test 6	5	3	5	3
Test 7	5	2	5	1
Test 8	5	3	5	0
Total	45	36	45	28
Percentage	80	62

and multiple lots) was taken from tables 3, 4, 5 (February cauliflower), and 7. The virus was maintained by repeated mechanical inoculation and aphid transmission. The virus from the same infected plants upon which the aphids had fed was mechanically inoculated into healthy plants. A comparison of the results obtained is given in table 6. It is evident that the three species of aphids were less efficient than mechanical inoculation in transmitting the virus: 89 to 90 per cent of the healthy cauliflower plants became infected when mechanically inoculated; 43 to 67 per cent when the virus was transmitted by aphids.

TABLE 5
TRANSMISSION OF CAULIFLOWER-MOSAIC VIRUS TO VARIETIES OF
CAULIFLOWER BY CABBAGE APHID (*BREVICORYNE BRASSICAE*)
AND GREEN PEACH APHID (*MYZUS PERSICAE*)

Variety of cauliflower	Cabbage aphid		Green peach aphid	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected
April.....	10	9	10	8
February.....	10	10	10	6
Danish Perfection.....	10	10	10	5
December.....	10	7	10	9
Dryweather Danish.....	10	8	20	9
Early March.....	10	6	10	1
Early Snowball.....	10	3	10	7
Extra Early Dwarf Erfurt.....	10	6	10	5
Hartmans Special Early.....	10	10	10	9
Hartmans Special Median.....	10	9	20	8
January.....	10	10	10	7
Late March.....	10	3	10	2
Late Pearl.....	10	6	10	9
November.....	10	10	10	8
St. Valentine.....	10	7	10	9
Super Snowball.....	10	9	10	4
February 759*.....	10	10	10	5
Christmas 2022*.....	20	16	20	8
Early March 713*.....	10	8	10	3
Early March 767*.....	10	9	10	7
Mission Special*.....	10	5	10	9
Total.....	220	171	240	138
Percentage.....	78	58

* The cauliflowers were grown from seeds from Ferry-Morse Seed Co., San Francisco.

TABLE 6
COMPARISON OF TRANSMISSION OF CAULIFLOWER-MOSAIC VIRUS BY
MECHANICAL INOCULATION WITH THREE SPECIES OF APHIDS

Number of plants from which virus was recovered	Mechanical inoculation			Aphids	Aphid transmission		
	Plants inoculated	Plants infected	Per cent infected		Plants inoculated	Plants infected	Per cent infected
27	125	112	90	Cabbage aphid, <i>Brevicoryne brassicae</i>	165	110	67
12	70	63	90	Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i>	70	30	43
20	120	107	89	Green peach aphid, <i>Myzus persicae</i>	120	69	58

RETENTION OF VIRUS

By Varying Numbers of Aphids with Daily Transfers. The retention of the virus was determined with three species of aphids reared on mosaic-infected cauliflower plants. In most tests, lots of 20 aphids were used; but occasionally 10, 5, and single aphids were employed. Each lot of aphids was transferred daily, usually for three days, to successive healthy cauliflower seedlings. In preliminary work the aphids sometimes were transferred daily

TABLE 7
RETENTION OF CAULIFLOWER-MOSAIC VIRUS BY THREE
SPECIES OF APHIDS

Aphid species and number of insects per lot	Number of lots	First day		Second day		Third day	
		Seedlings inoculated	Seedlings infected	Seedlings inoculated	Seedlings infected	Seedlings inoculated	Seedlings infected
Cabbage aphid, <i>Brevicoryne brassicae</i> :							
1 per lot.	3	18	4	18	0	18	0
5 per lot.	1	6	2	6	0	6	0
10 per lot.	1	7	7	7	0	7	0
20 per lot.	7	42	34	42	0	42	0
Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i> :							
20 per lot.	6	40	16	40	0	40	0
Green peach aphid, <i>Myzus persicae</i> :							
5 per lot.	2	10	2	10	0	10	0
20 per lot.	9	55	29	55	0	55	0

for 30 days to successive healthy cauliflower seedlings. These preliminary tests are not tabulated, but no transmissions were obtained after the first day.

As shown in table 7, each of the three species of aphids transmitted the virus from diseased to healthy cauliflower during the first day; but none of the lots tested produced infection during the second or third day.

By Lots of 20 Aphids with Hourly Transfers. An attempt was made to determine more precisely how long the cabbage, turnip, and green peach aphids retained the cauliflower-mosaic virus. Each of 3 lots of 20 wingless, mature aphids of each species, reared on mosaic-infected cauliflower plants, was transferred hourly to 10 successive healthy plants.

As table 8 shows, 2 of 3 lots of 20 cabbage aphids transmitted the virus during the first and second hours and 1 lot during the first hour only. Each lot of the other two species of aphids transmitted the virus during the first hour only.

By Single Aphids in Short Feeding Time. Previously noninfective, mature, wingless aphids were starved for a period of 30 minutes in a stentor dish, with the bottom covered with moist filter paper. Each aphid was transferred with a moistened camel's-hair brush from the moist chamber to a mosaic-infected

cauliflower plant and allowed to feed for 5 or 10 minutes. Some of the aphids crawled about on the leaf, but after finding a suitable feeding place they settled down, with the antennae parallel to the body and the labium at right angles to the leaf and touching it. Each aphid was observed through a hand lens, and when it did not immediately withdraw the stylets, the feeding time was taken.

TABLE 8
RETENTION OF CAULIFLOWER-MOSAIC VIRUS BY THREE SPECIES OF
APHIDS TRANSFERRED HOURLY TO 10 SUCCESSIVE
HEALTHY CAULIFLOWER SEEDLINGS

Aphid species and lot no.	Number of aphids on first plant	Results on successive plants, with hourly transfers*										Last infection produced by aphids, hour
		1st	2d	3d	4th	5th	6th	7th	8th	9th	10th	
Cabbage aphid, <i>Brassicorhynchus brassicae</i> :												
Lot 1.....	20	+	+	-	-	-	-	-	-	-	-	2d
Lot 2.....	20	+	+	-	-	-	-	-	-	-	-	2d
Lot 3.....	20	+	-	-	-	-	-	-	-	-	-	1st
Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i> :												
Lot 1.....	20	+	-	-	-	-	-	-	-	-	-	1st
Lot 2.....	20	+	-	-	-	-	-	-	-	-	-	1st
Lot 3.....	20	+	-	-	-	-	-	-	-	-	-	1st
Green peach aphid, <i>Myzus persicae</i> :												
Lot 1.....	20	+	-	-	-	-	-	-	-	-	-	1st
Lot 2.....	20	+	-	-	-	-	-	-	-	-	-	1st
Lot 3.....	20	+	-	-	-	-	-	-	-	-	-	1st
Total +.....	..	9	2	0	0	0	0	0	0	0	0
Total -.....	..	0	7	9	9	9	9	9	9	9	9

* The plus sign (+) indicates the production of the disease, and the minus (-) shows that no disease resulted.

The time occupied by each aphid in finding a suitable feeding place and settling down to feed, is referred to by Watson (1936) as the "penetration time," but probably should be designated as prepenetration time. The average prepenetration time was 5.1 minutes with the cabbage aphid, 3.6 minutes with the turnip aphid, and 4.7 minutes with the green peach aphid (table 9).

After feeding 5 or 10 minutes on a mosaic-infected cauliflower plant, each aphid was transferred to a healthy cauliflower seedling; and it was fed for 5 or 10 minutes. The aphid then was transferred to 5 more healthy cauliflower seedlings in succession and fed 10 minutes on each.

Three species of aphids were tested in this way. Table 9 shows the results with those aphids that transmitted the virus to at least one healthy cauliflower seedling. As this table shows, 25 cabbage aphids, 5 turnip aphids, and 5 green peach aphids, each tested singly, produced infections only in the first cauliflower seedling. One cabbage aphid after feeding 5 minutes on a diseased cauliflower seedling failed to infect the first two healthy cauliflower seedlings, but infected the third plant. One green peach aphid caused an infection of

TABLE 9
RETENTION OF CAULIFLOWER-MOSAIC VIRUS BY SINGLE APHIDS
TRANSFERRED AT 5- OR 10-MINUTE INTERVALS TO SIX
SUCCESSIVE HEALTHY CAULIFLOWER SEEDLINGS

Aphid species and insect no.	Time on diseased cauliflower, minutes		Results* on successive plants, with 10 minutes† per plant					
	Prepen- etration time	Feeding time	1st plant†	2nd plant	3rd plant	4th plant	5th plant	6th plant
Cabbage aphid, <i>Brevicoryne brassicae</i>:								
No. 1.....	10	5	-	-	+	-	-	-
No. 2.....	13	10	+	-	-	-	-	-
No. 3.....	11	10	+	-	-	-	-	-
No. 4.....	11	10	+	-	-	-	-	-
No. 5.....	11	10	+	-	-	-	-	-
No. 6.....	7	10	+	-	-	-	-	-
No. 7.....	7	10	+	-	-	-	-	-
No. 8.....	7	10	+	-	-	-	-	-
No. 9.....	6	10	+	-	-	-	-	-
No. 10.....	6	10	+	-	-	-	-	-
No. 11.....	6	10	+	-	-	-	-	-
No. 12.....	5	10	+	-	-	-	-	-
No. 13.....	5	10	+	-	-	-	-	-
No. 14.....	5	10	+	-	-	-	-	-
No. 15.....	5	10	+	-	-	-	-	-
No. 16.....	5	10	+	-	-	-	-	-
No. 17.....	5	10	+	-	-	-	-	-
No. 18.....	4	10	+	-	-	-	-	-
No. 19.....	4	10	+	-	-	-	-	-
No. 20.....	4	10	+	-	-	-	-	-
No. 21.....	4	10	+	-	-	-	-	-
No. 22.....	4	10	+	-	-	-	-	-
No. 23.....	3	10	+	-	-	-	-	-
No. 24.....	2	10	+	-	-	-	-	-
No. 25.....	2	10	+	-	-	-	-	-
No. 26.....	1	10	+	-	-	-	-	-
Total or average.....	5.1	..	25+, 1-	26-	1+, 25-	26-	26-	26-
Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i>:								
No. 1.....	7	10	+	-	-	-	-	-
No. 2.....	4	10	+	-	-	-	-	-
No. 3.....	3	10	+	-	-	-	-	-
No. 4.....	2	10	+	-	-	-	-	-
No. 5.....	2	10	+	-	-	-	-	-
Total or average.....	3.6	..	5+	5-	5-	5-	5-	5-
Green peach aphid, <i>Myzus persicae</i>:								
No. 1.....	7	5	+	-	+	-	-	-
No. 2.....	6	5	+	-	-	-	-	-
No. 3.....	6	5	+	-	-	-	-	-
No. 4.....	5	5	+	-	-	-	-	-
No. 5.....	3	5	+	-	-	-	-	-
No. 6.....	2	5	+	-	-	-	-	-
Total or average.....	4.7	..	6+	6-	1+, 5-	6-	6-	6-

* The plus sign (+) indicates the production of the disease, and the minus (-) shows that no disease resulted.
† The feeding time on the first plant was 5 minutes for cabbage aphid no. 1 and all green peach aphids; all other feeding periods on healthy plants were 10 minutes.

the first cauliflower plant, failed to infect the second, but infected the third. Negative results are not shown in table 9: 72 cabbage, 41 turnip, and 40 green peach aphids tested singly in short feeding periods failed to transmit the virus.

TABLE 10
LOSS AND RECOVERY OF INFECTIVITY BY APHIDS ON CAULIFLOWER
PLANTS INOCULATED WITH CAULIFLOWER-MOSAIC VIRUS

Aphid species and first inoculated plant no.	Results* when a lot of 20 aphids was transferred from the first inoculated plant to a second healthy plant on the following day											Days to first symptom on first inoculated plant	
	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th		
Cabbage aphid, <i>Brevicoryne brassicae</i> :													
Plant 1	-	-	+	+	+	+	+	+	+	+	-	18	
Plant 2	-	-	-	-	-	+	-	+	+	-	-	20	
Plant 3	-	-	-	-	-	-	-	+	-	-	+	21	
Plant 4	-	-	-	-	-	+	+	+	-	-	+	24	
Plant 5	-	-	-	-	-	-	+	+	+	+	+	25	
Plant 6	-	-	-	-	-	-	+	-	-	-	-	30	
Total +	0	0	1	1	1	3	4	5	3	2	3		
Total -	6	6	5	5	5	3	2	1	3	4	3		
Green peach aphid, <i>Myzus persicae</i> :													
Plant 7	-	-	-	+	-	-	-	-	-	-	+	30	
Plant 8	-	-	-	-	-	-	-	-	-	-	+	33	
Total +	0	0	0	1	0	0	0	0	0	0	2		
Total -	2	2	2	1	2	2	2	2	2	2	0		

The plus sign (+) indicates the production of the disease, and the minus (-) shows that no disease resulted.

RECOVERY OF VIRUS BY APHIDS FROM AN INOCULATED PLANT BEFORE SYMPTOMS DEVELOP

An attempt was made to determine whether the cabbage and green peach aphids were able to recover the virus from inoculated cauliflower plants before the first symptom of the disease developed. A large population of aphids reared on mosaic-infected cauliflower plants was transferred to a healthy cauliflower plant for 3 days. Each day from the fourth to the fourteenth day, one lot of 20 of these aphids was transferred from the plant so inoculated to a healthy cauliflower plant. Each lot of aphids remained on the second inoculated plant for 3 days and then the plant was fumigated to kill the aphids. The results reported in table 7 indicate that the aphids do not retain the virus longer than 1 day. Therefore the aphids were presumed to have lost their infectivity before transfer to the second healthy plant; and any transmission that occurred may be attributed to recovery of the virus from the first inoculated plant.

The transmissions by the two aphid species and also the incubation period of the disease, or the period for the earliest symptom (cleared veinlets) to develop in the original infected cauliflower plant, are shown in table 10. With the cabbage aphid, the elapsed time to the first recovery of the virus varied from 6 to 11 days. The incubation period of the disease in the original infected

cauliflower plants varied from 18 to 30 days. With the green peach aphid, the elapsed time to the first recovery of the virus was 7 days with one lot, 14 days with the other. The incubation periods of the disease in the original infected cauliflower plants were 30 and 33 days, respectively. Fourteen additional lots of aphids, not tabulated in table 10, failed to recover the virus from the fourth to fourteenth day.

The fact that no transmissions occurred on the fourth or fifth day supports the assumption that the aphids had lost the infectivity acquired on the original mosaic-infected plant before they were transferred to the second healthy plant. The results show that the aphids were able to recover the virus from the first inoculated plant before symptoms developed on it.

MECHANICAL INOCULATION WITH VIRUS EXTRACT FROM CRUSHED APHIDS

An attempt was made to transmit the cauliflower-mosaic virus with the centrifuged virus extract of crushed, infective cabbage aphids by mechanical inoculation of healthy cauliflower and annual stock plants. The centrifuged virus extract prepared from 10 grams of infective cabbage aphids crushed in 90 cc of sterile distilled water was inoculated in 10 healthy cauliflower seedlings and 10 annual stock plants. Four cauliflower and 8 annual stock plants were infected. In three other tests, 4.3, 4.0 and 0.68 grams of infective cabbage aphids were crushed in sterile distilled water. The results obtained with the virus extract inoculated into healthy cauliflower seedlings and annual stock plants were as follows: 4.3 grams, 20 plants inoculated remained healthy; 4.0 grams, 1 of 10 annual stock plants became infected, 10 cauliflower plants inoculated remained healthy; and 0.68 grams, 1 of 10 cauliflower seedlings became infected, 10 annual stock plants remained healthy. Of a total of 80 plants inoculated, 14, or 18 per cent, became infected.

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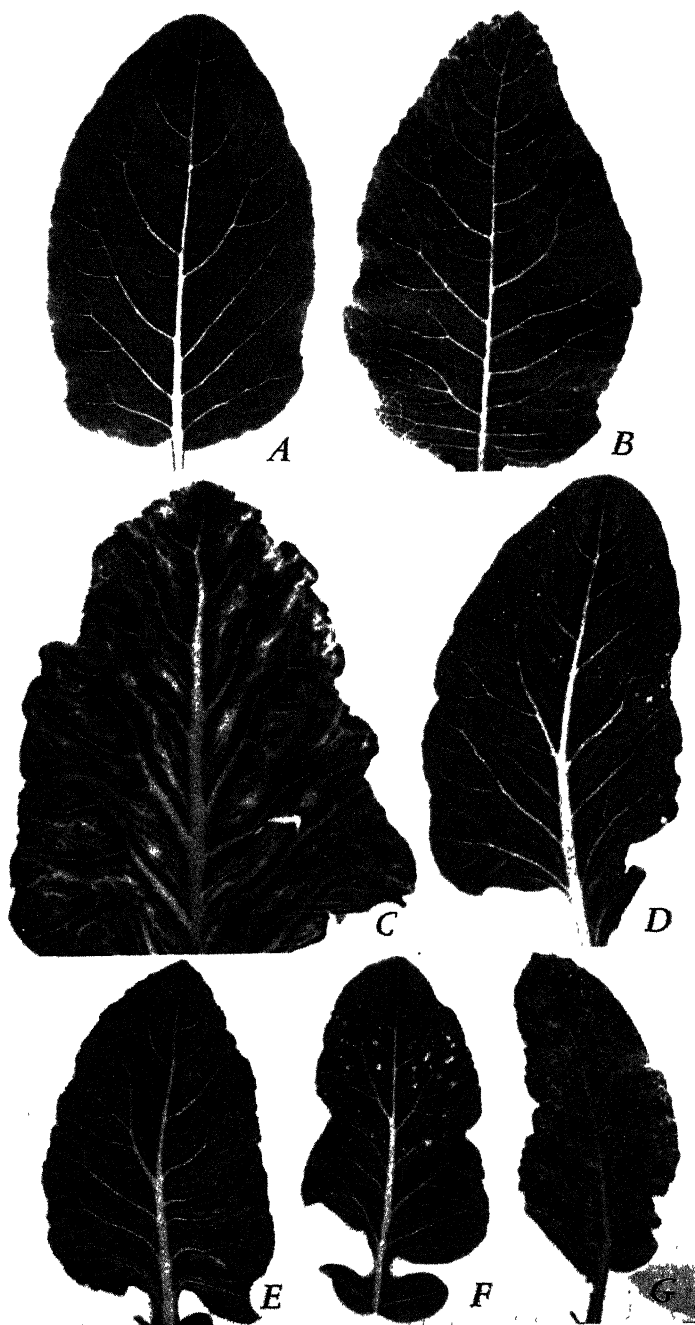


Plate 1. Symptoms of cauliflower mosaic on leaves of cauliflower (*Brassica oleracea* var. *botrytis*): A, leaf from healthy check or control plant; B, vein clearing; C, veinbanding, mottling, and distortion; D, curvature of the midrib; E, F, necrotic lesions; G, necrotic spotting, curvature of the midrib, and distortion.

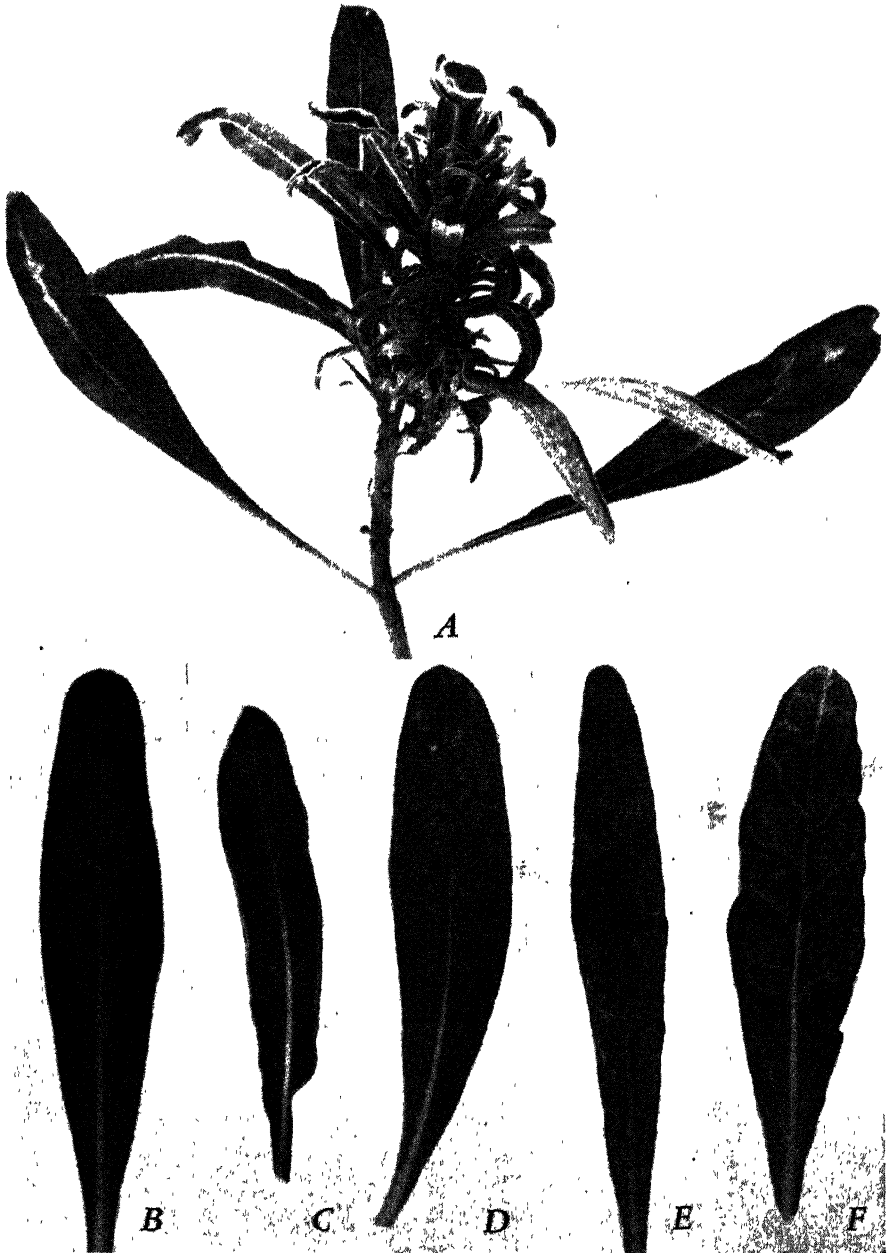


Plate 2. Symptoms of cauliflower mosaic on stock plants (*Matthiola incana* var. *annua*): A, plant infected by means of green peach aphid (*Myzus persicae*) showing shortened internodes and secondary shoots; B, leaf from healthy check or control plant; C, clearing of veins; D, leaf from plant infected with cabbage aphid (*Brevicoryne brassicae*) showing circular chlorotic areas; E, mottling; F, protruding veins on lower surface of leaf.

**THE MOST IMPORTANT SPECIES OF APHIDS
ATTACKING CRUCIFEROUS CROPS
IN CALIFORNIA**

E. O. ESSIG

THE MOST IMPORTANT SPECIES OF APHIDS ATTACKING CRUCIFEROUS CROPS IN CALIFORNIA¹

E. O. ESSIG²

APHIDS cause great damage to cruciferous crops—broccoli, brussels sprouts, cabbage, cauliflower, kale, mustard, radishes, turnips, and others. These insects weaken, stunt, and sometimes even kill the plants by sucking the juice. They may make cabbage, brussels sprouts, cauliflower, and broccoli wholly unfit to market, for it is difficult or impossible to remove them from the heads of such plants. On seed farms, they may completely destroy the plants before harvest by infesting the seedstalks. They cause even greater losses by transmitting plant viruses, which may destroy the plants over considerable areas.

Three aphid species that breed on these plants are responsible for most of the damage in California. These are the cabbage aphid, *Brevicoryne brassicae* (Linnaeus); the turnip or false cabbage aphid, *Rhopalosiphum pseudo-brassicae* (Davis); and the green peach aphid, *Myzus persicae* (Sulzer). All three species have become world wide in distribution and are to be found generally throughout the ranges of the host plants. This paper assembles the salient facts on their synonymy, characteristics, life histories, distribution, and host plants, as a basis for studies on their transmission of viruses and on their control.

THE CABBAGE APHID

Brevicoryne brassicae (Linnaeus) (Van der Goot, 1915, 1918)³ *

Aphis brassicae Linnaeus (1746, 1758)⁴

Aphis raphani Schrank (1801)⁵

Aphis insatidis Boyer de Fonscolombe (1841)⁷

Aphis floris-rapae Curtis (1860, p. 69-83)⁸

The cabbage aphid, *Brevicoryne brassicae* (Linnaeus) is usually more abundant on cruciferous crops than any other aphid and is therefore more injurious.

The cabbage aphid can be distinguished from other aphids by the large closely crowded colonies (fig. 1), the white waxy powdery covering over the bodies of the alate and apterous individuals, and the cruciferous host plant.

¹ Paper received for publication June 20, 1947.

² Professor of Entomology and Entomologist in the Experiment Station.

³ See "Literature Consulted" for citations, referred to in the text by author and date.

⁴ The genus *Brevicoryne* was proposed by B. Das and erected by Van der Goot in 1915 (1915 and 1918).

⁵ First referred to by Linnaeus in 1746 (1746) and described by him in 1758 (1758).

⁶ A synonym erected by Schrank (1801) for an aphid feeding on cabbage in Bavaria, Germany.

⁷ A synonym collected in the Province of Aix, France, previous to 1841, was described by Boyer de Fonscolombe (1841).

⁸ This species was described by Curtis in 1860 (1860, p. 69-83) and called the turnip-flower plant louse. In this article Curtis lists the suggested control measures (tobacco decoctions, lime dust, hand-picking infested parts of plants) and discusses at length the insect predators and parasites of this aphid.

The turnip aphid has often been confused with this species; differences are discussed on pages 412-13.

Mounted specimens are readily distinguished by the long antennal segment III, which in the alates is covered with circular secondary sensoria; by the very short cornicles; the long slender tarsi; and the unguis, spur, or filament of the terminal antennal segment, which is four to five times as long as the base. Figures 2 and 3 show details of the male and three forms of females.



Fig. 1.—The cabbage aphid, *Brevicoryne brassicae* (Linn.): a typical colony on the underside of a cabbage leaf. The white powdery waxy covering is plainly shown on many individuals. ($\times 4$)

Life History. In the warmer climates of the distribution of the cabbage aphid, there is continuous reproduction by parthenogenesis, and only apterous and alate parthenogenetic females occur. There may be as many as 30 or more generations during the year, so that extremely dense and destructive populations are built up. During the winter these populations may be slowed up and even reduced by cooler temperatures, rain, and other climatic factors. In California this aphid is found in prosperous colonies on wild mustard (*Brassica campestris*) and other native or weedy cruciferous plants. In summer and fall, many of the natural host plants disappear and the aphid is forced to cultivated crops, which may suffer severely from its attacks. Wherever summer rains occur, the native hosts continue to serve as reservoirs for feeding and breeding.

During spring—April and May—and fall—October and November—great numbers of winged migrants may be seen in California. They literally fill the air in certain areas along the foothills.

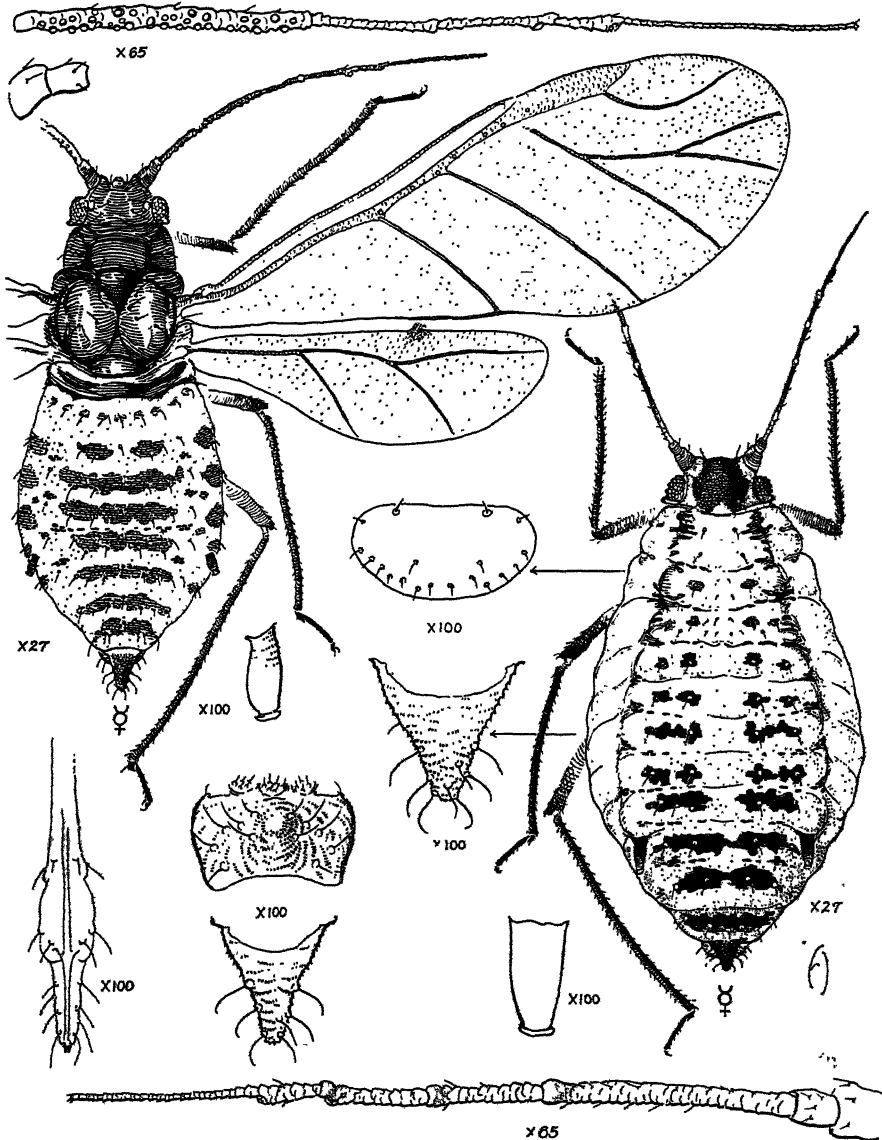


Fig. 2.—The cabbage aphid, *Brevicoryne brassicae* (Linn.): the alate and apterous viviparous females. The important body structures, including antennae, cornicles, anal plate, cauda, and tip of rostrum, used in identification, are greatly enlarged as indicated on the illustration. Note the long antennal segment III covered with sensoria and the long slender tarsi, which separate this aphid from others feeding on cruciferous plants. (Drawing by Frieda Abernathy.)

In the more northern reaches of its distribution, the spring and summer reproductives (migrants and alienicolae) are replaced in fall by sexuparae, or gynoparae. The sexuparae are apterous and alate viviparous parthenogenetic females that give birth to sexuales. Males and females of the sexuales (fig. 3)

<i>Brassica napobrassica</i>	<i>Cardamine hirsuta</i>	<i>Mathiola incana</i>
<i>Brassica napus</i>	<i>Cochlearia anglica</i> (C.	<i>Mimulus guttatus</i>
<i>Brassica nigra</i>	arctica)	<i>Myagrum perfoliatum</i>
<i>Brassica oleracea</i>	<i>Crambe</i> sp.	<i>Raphanus landra</i>
<i>Brassica oleracea</i> var.	<i>Diplotaxis tenuifolia</i>	<i>Raphanus maritimus</i>
acephala	<i>Eruca sativa</i>	<i>Raphanus raphanistrum</i>
<i>Brassica oleracea</i> var.	<i>Erucastrum obtusangulum</i>	<i>Raphanus sativus</i> var.
gemmifera	<i>Erysimum canescens</i>	longipinnatus
<i>Brassica pekinensis</i>	<i>Iberis</i> sp.	<i>Sinapis juncea</i> var. napi-
<i>Brassica rapa</i>	<i>Isatis tinctoria</i>	formis (<i>Brassica napi-</i>
<i>Bunias erucago</i>	<i>Lepidium amplexicaule</i>	formis)
<i>Cakile maritima</i> (C.	<i>Lepidium graminifolium</i>	<i>Sisymbrium officinale</i>
edentula)	<i>Lepidium ruderales</i> [sic]	<i>Sisymbrium sophia</i>
<i>Capsella bursa-pastoris</i>	<i>Lepidium sativum</i>	
<i>Capsicum frutescens</i> (C.	<i>Lunaria annua</i>	
annuum)	<i>Mathiola bicornis</i>	

Some other plants reported as hosts are very questionable. They may have been only resting places for the dispersing or migrating alates.

Origin and Distribution. The cabbage aphid is one of the commonest species to be found throughout the temperate and subtropical regions of the world. This wide distribution has no doubt been made possible by the very extensive distribution and abundance of its cruciferous host plants. The many vegetables, ornamental flowering plants, and economic weeds have been carried through commerce to all inhabited lands and have become adapted in all except the most extreme climates.

The cabbage aphid has no doubt been associated with cultivated cruciferous crops in certain areas ever since they were developed by man. Its exact place of origin may never be definitely established. However, this insect appears to have first been associated with host plants originating in the Palaearctic Region and was early reported on wild and cultivated plants in Europe. It probably occurred on cabbages and related host plants long before it was recorded in print. Frisch (1734) is credited as having first brought this aphid to the attention of the public in 1734 when he reported it from Germany, described its work, and presented drawings that aid in its identification.

So far as I am able to ascertain, the cabbage aphid does not appear to be a serious pest of cruciferous crops in Asia. It is rarely found in lists of destructive insects from that continental area. Wu (1935) in his *Catalogus Insectorum Sinensium* does not list this species. Recently Ying-Tou Mao^{*} reviewed Chinese literature on aphids thoroughly, but found the cabbage aphid reported only from Hangchow, Fukien, and Taiwan. This species was not included in any of several large collections of aphids I have received from China. The shortage of records may simply indicate a lack of intensive study of this insect in China. Still, it is especially significant in view of the fact that many of the most important and useful members of the cabbage family originated in that country. It may be a further indication of the possible origin of the cabbage aphid in Northwestern Europe, the home of the cabbage.

The species does appear to be quite widely distributed in Japan and has been reported by many entomologists in that country.

From the information at hand, it appears possible that the cabbage aphid

^{*} Ying-Tao Mao. A list of Chinese aphids and their host plants. Typewritten manuscript.

may have originated in Western Europe in association with wild or sea cabbage, charlock, cabbage, cauliflower, brussels sprouts, kale, and other wild and cultivated cruciferous plants. Its counterpart in Asia and the Pacific islands appears to be the turnip aphid.

It is hardly practical to list all or even the larger geographical units throughout the world, especially in Africa and South America, where the cabbage aphid has been found. The following list has been compiled from many sources:

Asia: Astrakhan, Bessarabia, China (Ainoy, Hangchow, Hopei, Kiangsu, Taiwan), Iraq, India (Lahore), Japan (Fukuoka, Hokkaido, Morioka), Palestine, Siberia, Syria, Transcaucasia

Africa: Bengal, Cape of Good Hope, Egypt, Eritrea, Kenya, Madagascar, Mauritius, Morocco, Nairobi, Natal, Nyassaland, Orange Free State, Rhodesia, Transvaal

Australia: Queensland, New South Wales, South Australia, Tasmania

New Zealand

Europe: Belgium, Czechoslovakia, France, Germany, Great Britain, Holland, Ireland, Italy, Lettland, Malta, Norway, Poland, Serbia, Spain, Sweden, U.S.S.R.

North America: Canada (British Columbia, Ontario, Quebec, and other provinces), United States (every state), West Indies (Cuba, Puerto Rico, Santo Domingo), Bermuda, Guatemala, Mexico

South America: Argentina, Brazil, Chile, Colombia, Virgin Islands

South Pacific: Fiji, Hawaii

THE TURNIP APHID¹⁰

Rhopalosiphum pseudobrassicae (Davis)

Aphis pseudobrassicae Davis (1914, p. 231)

Lipaphis pseudobrassicae (Davis) (Mordvilko, 1928, p. 200)

Aphis mathiellae Theobald (1918) (Hall, 1926, p. 24)

The turnip or false cabbage aphid is almost as destructive to cruciferous crops as is the cabbage aphid. It appears to have originated in Asia, where it has a wide distribution. It has apparently been introduced into many other countries and has become widely distributed in many localities.

It was no doubt early confused with the cabbage aphid and became firmly established in most areas before it was recognized as a distinct species. In fact, its true identity was not discovered until 1914 when it was described as *Aphis pseudobrassicae* by Davis (1914, p. 231) from specimens collected by W. J. Schoene on cabbage at Geneva, New York on July 15, 1912, and on mustard and kale taken at Evansville, Indiana, November 20 of the same year. In September and October, 1913, additional material was taken on radish and turnip at Lafayette, Indiana, and on turnip at College Station, Texas, by F. B. Paddock (1915).

In size and general appearance it greatly resembles the cabbage aphid. However, in California, it seems to have less powdery wax on its body. Specimens of whole colonies over large areas may appear bright green and almost devoid of the white waxy secretion so characteristic of the cabbage aphid. However, pulverulent forms do occur here. Specimens are paler in color than the cabbage aphid and lack the broad transverse broken dark bands on the dorsum of the alates and apterous forms; antennal segment III is much

¹⁰ This common name has also been used for the cabbage aphid, *Brevicoryne brassicae* (Linn.). The turnip aphid is also known as the false cabbage aphid.

shorter; tarsi are shorter; cornicles are longer; there are secondary sensoria present on antennal segment III and IV of the alates; the unguis, spur, or filament of the terminal antennal segment is three times the length of the base; and the cauda is triangular in shape. Important characters for identification are shown in figure 4.

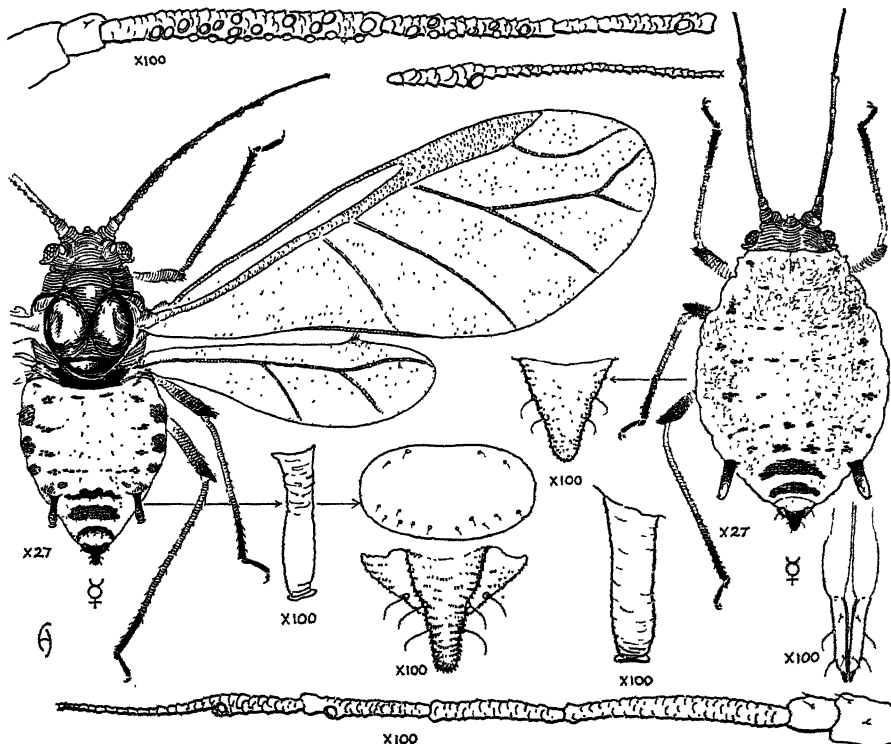


Fig. 4.—The turnip or false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis): alate and apterous viviparous females with the antennae, cornicles, anal plates, caudas, and tip of rostrum greatly enlarged as indicated. Note the sensoria on both antennal segments III and IV of the alate. (Drawings by Frieda Abernathy.)

Life History. The life history is similar to that of the cabbage aphid. Allen and Harrison (1941) have studied the life history in the south and state that there are 15 to 46 generations a year; 50 to 100 young are produced by a single female; aphids live as long as 2 months; no sexuales appear in the south; living forms occur throughout the year; most damage is done during the winter months—October to and including the following March. The life history in the northern limits of distribution has not been studied and, although sexual forms are probably produced, they have not been reported.

Host Plants. The host plants are similar to those of the cabbage aphid, but are as yet not so well known. The following are reported to date:

Barbarea vulgaris
Brassica cauloxypa
Brassica oleracea

Brassica juncea (*B.*
rugosa)
Brassica kaber (*B.*
campestris)

Brassica napobrassica
Brassica napus
Brassica nigra

<i>Brassica oleracea</i>	<i>Chrysanthemum</i>	<i>Nasturtium officinale</i>
<i>Brassica oleracea</i> var.	<i>coronarium</i> (?)	(<i>Roripa nasturtium</i>)
<i>acephala</i>	<i>Descurainia sophia</i>	<i>Phaseolus</i> sp. (?)
<i>Brassica oleracea</i> var.	<i>Gynandropsis speciosa</i> (?)	<i>Ranunculus sceleratus</i> (?)
<i>botrytis</i>	<i>Lactuca sativa</i>	<i>Raphanus raphanistrum</i>
<i>Brassica oleracea</i> var.	<i>Lepidium campestre</i>	<i>Raphanus sativus</i>
<i>capitata</i>	<i>Lepidium virginicum</i>	<i>Raphanus sativus</i> var.
<i>Brassica pekinensis</i>	<i>Lycopersicon esculen-</i>	<i>longipinnatus</i>
<i>Brassica rapa</i>	<i>tum</i> (?)	<i>Thlaspi arvense</i>
<i>Capsella bursa-pastoris</i>	<i>Mathiola incana</i>	

Distribution. As previously indicated, this aphid probably originated in Asia and has spread by commerce throughout many other parts of the world. So far no records of its occurring in Europe have been noted in publications, which seems quite remarkable. The following distribution is known:

Asia: China (Chekiang, Hopei, Kiangsu, Kwangtung, Shantung, Taiwan), Korea (Sui-gan), India, Iraq, Japan (general, Daito Jima), Java, Loochoo, Siam, Sumatra

Africa: Egypt, Cape Colony, Maroc, Uganda

Australia: New South Wales

New Zealand

North America: Canada (British Columbia, Manitoba, Ontario, Quebec); United States (throughout much of the entire country: actually reported from Alabama, California—Berkeley, El Centro, Half Moon Bay, Lompoc, Los Angeles, Riverside, San Francisco, Stanford University, Stockton, Ventura—Connecticut, Florida, Georgia, Illinois, Indiana, Louisiana, Maine, Maryland, Mississippi, New Jersey, New York, North Carolina, Ohio, Pennsylvania, South Carolina, Tennessee, Texas, Virginia, Wisconsin), Bermuda, Puerto Rico

South America: Argentina, Trinidad

South Pacific: Hawaii (Hawaii, Oahu)

THE GREEN PEACH APHID¹¹

Myzus persicae (Sulzer)
Aphis persicae Sulzer (1776, p. 105)
Aphis dianthi Schrank (1801)
Aphis vulgaris Kyber (1815)
Aphis furcipes Rafinesque (1817)
Aphis rapae Curtis (1842)
Aphis vastator Smea (1846)
Aphis cyanoglossi Walker (1848)
Aphis egressa Walker (1849)
Aphis redundans Walker (1849)
Aphis aucta Walker (1849)

Aphis persicaecola Boisduval (1867)
Siphonophora achyranthes Monell (1879)
Rhopalosiphum tulipae Thomas (1879)
Myzus malvae Oestlund (1886)
Myzus pergandii Sanderson (1901)
Phorodon cyanoglossi Williams (1910)
Rhopalosiphum solani Theobald (1912)
Rhopalosiphum betae Theobald (1913)
Rhopalosiphum lactucellum Theobald (1915)
Rhopalosiphum tuberosellae Theobald (1919)

The green peach aphid is without doubt the most important economic species in the entire family Aphididae. It is not only cosmopolitan in distribution and feeds on more varieties of host plants, but it is also capable of transmitting more kinds of plant viruses than any other insect known at the present time.

Description and Life History. Like most widely distributed aphids, the green peach aphid has a variable life history, not greatly different from that of the cabbage aphid. In the warmer tropical and subtropical areas, it is maintained by continuous generations of viviparous parthenogenetic fe-

¹¹ This aphid is called the tobacco aphid in Southern Rhodesia and other parts of Africa (Brain, 1940, p. 254).

males—both winged and wingless. There may be 30 to 40 generations a year, although the complete life history has not been accurately recorded in all areas. In the northern limits of its range, it is maintained chiefly by migrations from more favorable and warmer areas where it has persisted and multiplied even during the winter. The migrations or dispersals northward begin early in February, March, April, and May, and may continue until winter approaches. The advance northward is regulated by the increasingly favorable seasonal weather conditions. Escapes from greenhouses and even residences may also account for small isolated colonies which may appear in northern regions in advance of the regular migrations.

In quite cold northern climates the green peach aphid may give rise to sexuales, and eggs are produced that survive the winters and give rise to spring generations. The alternate winter hosts are usually fruit trees, including apricots, cherries, nectarines, peaches, and plums.

In California sexual forms are rarely taken. But males and females were collected on sand cherry, *Prunus pumila*, at Riverside by R. C. Dickson on December 20, 1940. They are probably quite common but are not readily discovered.

The various forms are:

Stem mother, a pink form that hatches from the overwintering egg and gives rise to succeeding generations.

Apterous viviparous female (fig. 5, B), a pale yellow or green form born from the stem mother and living on the primary host. She gives birth to winged spring migrants.

Spring migrants, greenish, yellowish, or reddish, black-marked winged viviparous females (fig. 5, A) that migrate from the winter primary hosts and settle on spring and summer hosts of all kinds. These may also migrate great distances, especially if carried by favorable winds. The apterous females are usually greenish and have the apical portions of the antennae and legs, and tips of cornicles dusky or black. The alates are yellowish or greenish, with the head, thorax, most of the antennae, apical portions of leg segments, bases of the cornicles, lateral spots, and a large median dorsal spot on the abdomen black. The swollen cornicles and black dorsal abdominal spot serve to identify this aphid readily.

Summer alate and apterous viviparous females are not unlike the spring migrants. They are produced through many generations on the summer hosts and disperse freely over wide areas. According to Profft (1939, p. 14-15) winged adults have been found on islands 36 miles from the North Sea coast of Germany and on Spitzbergen, hundreds of miles from their normal habitat. The progeny of these may survive the winters in favorable areas.

Fall migrants, usually darker specimens that migrate to the primary host plants, where they mate and give rise to alate males and apterous sexual females. The latter lay the overwintering eggs.

Males, small, very dark, almost wholly black.

Oviparous females, apterous, not greatly different from the apterous viviparous females.

Host Plants. It will probably never be possible to secure an all-inclusive list of the host plants of this aphid. Its feeding habits are so varied that its

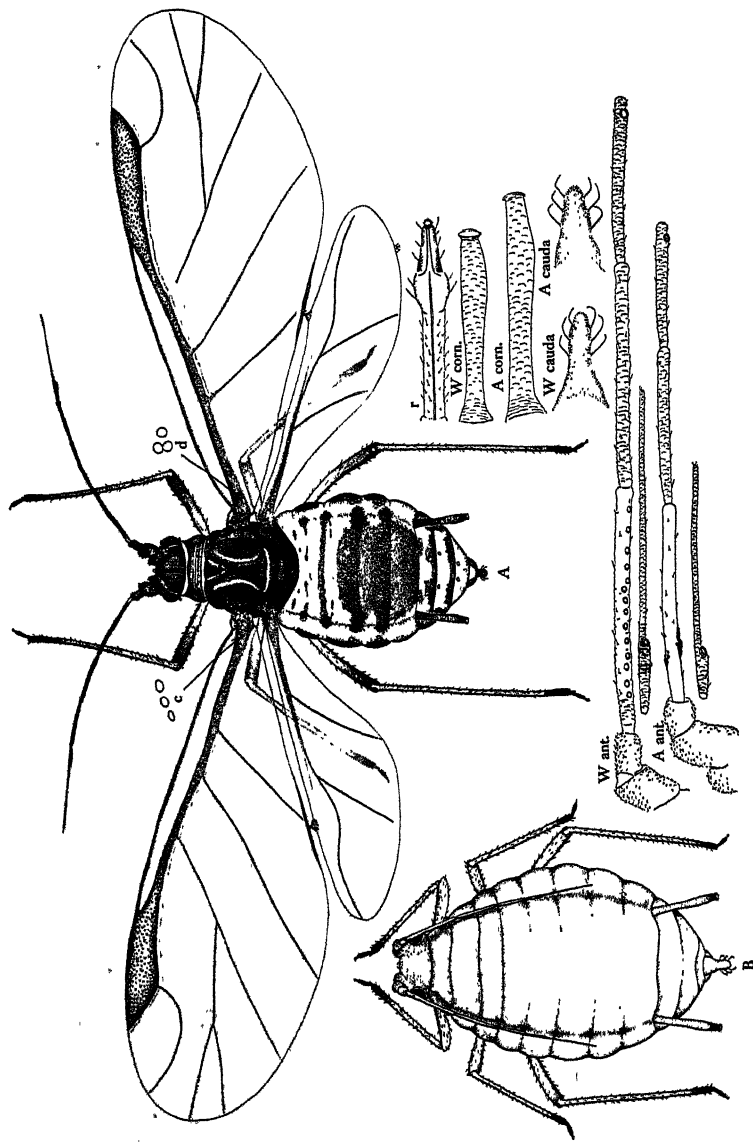


Fig. 5.—The green peach aphid, *Myzus persicae* (Sulzer): A, Adult alate viviparous female: c and d, fenestras or small transparent pores at the bases of the forewings; r, rostrum; W ant., antenna; W corn., cornicle; W cauda, cauda. B, Adult apterous viviparous female: A ant., antennae; A cauda, cauda. All greatly enlarged.

capacity for acquiring new hosts seems to be unlimited. The following list is as complete as facilities at hand permit:

<i>Abutilon</i> sp.	<i>Capsicum dulce</i>	<i>Cyclamen europaeum</i>
<i>Acalypha boemerioides</i>	<i>Capstium frutescens</i>	<i>Cyclamen indicum</i>
<i>Acanthus spinosus</i>	(<i>C. annuum</i>)	<i>Cynara cardunculus</i>
<i>Acer negundo</i>	<i>Carduus</i> sp.	<i>Cynoglossum grande</i>
<i>Acer nuttali</i> [sic]	<i>Carica papaya</i>	<i>Cyrtanthus</i> sp.
<i>Achyranthes</i> sp.	<i>Carthamus tinctorius</i>	<i>Cytisus</i> sp.
<i>Aconida cannabina</i> (<i>A. cuspidata</i>)	<i>Catalpa speciosa</i>	<i>Dalbergia sissoo</i>
<i>Ageratum conyzoides</i>	<i>Centaurea</i> sp.	<i>Daphne</i> sp.
<i>Alternanthera</i> sp.	<i>Centranthus ruber</i>	<i>Datura stramonium</i>
<i>Althaea rosea</i>	<i>Cerastium semidecandrum</i>	(<i>D. tatula</i>)
<i>Amaranthus</i> spp.	<i>Cestrum fasciculatum</i> var. <i>newellii</i>	<i>Daucus carota</i>
<i>Ammannia</i> sp.	<i>Cestrum pseudoquina</i>	<i>Dianthus caryophyllus</i>
<i>Amsinckia spectabilis</i>	<i>Chaerophyllum aromaticum</i>	<i>Dianthus chinensis</i>
<i>Anthemis cotula</i>	<i>Chaerophyllum hirsutum</i>	<i>Digitalis lutea</i>
<i>Antirrhinum majus</i>	<i>Chaerophyllum roseum</i>	<i>Digitalis purpurea</i>
<i>Apium graveolens</i>	<i>Cheiranthus cheiri</i>	<i>Dipsacus fullonum</i>
<i>Aquilegia canadensis</i>	<i>Chenopodium album</i>	<i>Dyssodia</i> sp.
<i>Aquilegia vulgaris</i>	<i>Chenopodium murale</i>	<i>Duranta repens</i> (<i>D. plumieri</i>)
<i>Arctium lappa</i>	<i>Chenopodium viride</i> [sic]	<i>Echinops echinatus</i>
<i>Arctium majus</i>	<i>Chrysanthemum balsamita</i>	<i>Emilia sonchifolia</i>
<i>Asclepias speciosa</i>	<i>Chrysanthemum coccineum</i>	(<i>Senecio sonchifolius</i>)
<i>Asparagus officinalis</i>	<i>Chrysanthemum frutescens</i>	<i>Erigeron canadensis</i>
<i>Asparagus plumosus</i>	<i>Chrysanthemum indicum</i>	<i>Erodium botrys</i>
<i>Asparagus sprengeri</i>	<i>Cichorium endivia</i>	<i>Erodium cicutarium</i>
<i>Astragalus</i> sp.	<i>Citrullus vulgaris</i>	<i>Eruca sativa</i>
<i>Atriplex</i> sp.	<i>Citrus aurantium</i>	<i>Erythronium dens-canis</i>
<i>Atropa belladonna</i>	<i>Citrus limonia</i>	<i>Escallonia pulverulenta</i>
<i>Aubrieta</i> sp.	<i>Citrus maxima</i>	<i>Euonymus communis</i>
<i>Barbarea vulgaris</i>	<i>Citrus medica</i>	<i>Euphorbia helioscopia</i>
<i>Bauhinia variegata</i>	<i>Clarkia elegans</i>	<i>Euphorbia pulcherrima</i>
<i>Bellis perennis</i>	<i>Clarkia pulchella</i>	<i>Ficus pumila</i>
<i>Bellis silvestris</i>	<i>Cnicus</i> sp.	<i>Foeniculum vulgare</i>
<i>Beloperone</i> sp.	<i>Cochlearia armoracia</i> (<i>Nasturtium armoracia</i>)	<i>Fragaria chiloensis</i>
<i>Beta vulgaris</i> (<i>B. bengalensis</i>)	<i>Codiaeum</i> sp. (<i>Croton</i> sp.)	<i>Freesia</i> sp.
<i>Bougainvillea campestris</i>	<i>Colocasia</i> sp.	<i>Fuchsia coccinea</i>
<i>Bougainvillea juncea</i>	<i>Convolvulus arvensis</i>	<i>Fuchsia macrantha</i>
<i>Bougainvillea spectabilis</i>	<i>Convolvulus crispus</i>	<i>Fuchsia magellanica</i> var. <i>globosa</i>
<i>Brassica kaber</i> (<i>B. arvensis</i>)	(<i>Ipomoea crispa</i>)	<i>Galactites tomentosa</i>
<i>Brassica napus</i>	<i>Coprosma baueri</i>	<i>Galium mollugo</i>
<i>Brassica nigra</i>	<i>Cordylus</i> sp.	<i>Geranium molle</i>
<i>Brassica oleracea</i>	<i>Coronopus didymus</i>	<i>Geranium robertianum</i>
<i>Brassica pekinensis</i>	<i>Cratogeomys</i> sp.	<i>Gladiolus</i> sp.
<i>Brassica rapa</i>	<i>Crepis tectorum</i>	<i>Gloxinia digitaliflora</i>
<i>Buddleia madagascariensis</i>	<i>Crocus</i> sp.	<i>Glycine</i> sp.
<i>Buddleia orientalis</i> [sic]	<i>Crotalaria laburnifolia</i>	<i>Gnaphalium spathulatum</i>
<i>Calceolaria</i> sp.	<i>Crotalaria mucronata</i>	<i>Godetia amoena</i>
<i>Calendula arvensis</i>	<i>Cryptostemma calendulaceum</i>	<i>Gossypium herbaceum</i>
<i>Calendula officinalis</i>	<i>Cucumis melo</i>	<i>Grindelia robusta</i>
<i>Camellia japonica</i>	<i>Cucurbita maxima</i>	<i>Hedera helix</i>
<i>Canna indica</i>	<i>Cucurbita moschata</i>	<i>Helianthus annuus</i>
<i>Capsella bursa-pastoris</i>	<i>Cucurbita pepo</i>	<i>Helichrysum bracteatum</i>
		<i>Heliotropium arborescens</i>
		(<i>H. peruvianum</i>)
		<i>Hemerocallis</i> sp.

Hibiscus abelmoschus (H. moschatus, *Abelmoschus moschatus*)

Hibiscus esculentus

Hibiscus roseus

Hordeum sp.

Humulus lupulus

Hyacinthus orientalis

Hydrangea sp.

Ilex sp.

Ionidium concolor

Ipomoea batatas

Ipomoea maxima [sic]

Ipomoea purpurea (*Convolvulus major*)

Iresine lindenii

Iris sp.

Justicia alba [sic]

Kalanchoë sp. (*Bryophyllum* sp.)

Kleinia neriifolia

Lactuca oldhamii

Lactuca sativa

Lactuca scariola

Lactuca spicata

Lamium sp.

Lantana sp.

Lathyrus odoratus
(*Pisum odorata*)

Lavatera assurgentiflora

Lepidium draba

Ligustrum vulgare

Lilium candidum

Lilium longiflorum

Linaria sp.

Liriodendron tulipifera

Lupinus termis

Lycopersicon esculentum
(*Solanum lycopersicum*)

Malus communis
(*Pyrus malus*)

Malva parviflora

Malva rotundifolia

Malvastrum coccineum

Markhamia platyacalyx

Marsilea quadrifolia

Marsilea vestita

Matricaria inodora

Mathiola sp.

Maurandia hendersonii [sic]

Mazus sp.

Melianthus major

Melilotus indica

Mentha aquatica (*M. hirsuta*)

Mercurialis annua

Mesembryanthemum sp.

Mimulus sp.

Montia perfoliata

Moraea iridioides

Myosotis scorpioides

Myrtus sp.

Narcissus sp.

Nasturtium armoracea

Nasturtium indicum

Nasturtium officinale (*Radicula nasturtium-aquaticum*, *Roripa nasturtium*)

Nemesia strumosa

Nemophila heterophylla

Nerium indicum (*N. odorum*)

Nerium oleander

Nicotiana rustica

Nicotiana tabacum

Onopordum acanthium

Opuntia sp.

Orobancha sp.

Orthocarpus erianthus

Oxalis cernua

Oxalis corniculata

Oxalis rosea

Panax lancasteri [sic]

Papaver somniferum

Parthenium argentatum

Pastinaca sativa

Paulownia sp.

Penstemon spectabilis

Requeria trinervia [sic]

Petasites tricholobus

Petunia hybrida

Pharbitis nil

Phaseolus vulgaris

Philadelphus coronarius

Physalis virginiana

Picris echioides

Pimelea sp.

Pimenta officinalis

Pisum sativum

Pisum sativum var. *arvense*

Pittosporum eugenioides

Pittosporum tobira

Pittosporum undulatum

Plantago sp.

Poa sp.

Polygonum hydropiper

Polygonum multiflorum

Polygonum persicaria

Portulaca oleracea

Primula forbesii

Primula polyantha

Primula vulgaris

Prunella vulgaris

Prunus americana

Prunus amygdalus

(*P. communis*)

Prunus armeniaca

Prunus avium

Prunus besseyi

Prunus cerasus

Prunus domestica

Prunus domestica var. *insititia*

Prunus mume

Prunus persica

Prunus serotina

Prunus virginiana

Prunus virginiana var. *melanocarpa*

Psidium guajava

Pulicaria dysenterica

(*Inula dysenterica*)

Quamoclit lobata

Radicula curvisiliqua

Ranunculus asiaticus

Ranunculus bulbosus

Raphanus raphanistrum

Raphanus sativus

Rapistrum rugosum

Rheum raphonticum

Ribes odoratum

Richardia africana

Ricinus communis

Rosa sp.

Rudbeckia laciniata

Rumex dentatus

Sagina subulata

Salix sp.

Salsola kali (*S. tragus*)

Salvia leucantha

Salvia mellifera

Sambucus canadensis

Sanguisorba officinalis

Sanicula menziesii

Saxifraga splendens [sic]

Secale cereale

Sedum artissimum

Senebiera pinnatifida

Senecio alpestris (*S. crassifolius*)

Senecio cruentus

Senecio elegans

Senecio jacobaea

Senecio mikanioides

Senecio renifolius

(*Cineraria renifolia*)

Senecio vulgaris

Sesamum orientale

Setaria viridis

Silene sp.

Sisymbrium canescens

Sisymbrium irio

Solanandra grandiflora

Solanum carolinense

Solanum dulcamara

Solanum melangena

<i>Solanum nigrum</i>	<i>Tragopogon</i> sp.	<i>Valerianella olitoria</i>
<i>Solanum pseudo-capsicum</i>	<i>Tribulus terrestris</i>	(<i>Valeriana olitoria</i>)
<i>Solanum tuberosum</i>	<i>Trifolium pratense</i>	<i>Verbena chamaedryfolia</i>
<i>Sonchus asper</i>	<i>Trifolium repens</i>	<i>Viburnum opulus</i>
<i>Sonchus oleraceus</i>	<i>Triticum aestivum</i> (<i>T. sativum</i> , <i>T. vulgare</i>)	<i>Vicia</i> sp.
<i>Spinacia oleracea</i>	<i>Tropaeolum majus</i>	<i>Vinca major</i>
<i>Stellaria aquatica</i>	<i>Tulipa</i> spp.	<i>Vinca minor</i>
<i>Stellaria media</i>	<i>Typha</i> sp.	<i>Viola odorata</i>
<i>Stizolobium deeringianum</i>	<i>Ulmus procera</i> (<i>U. campestris</i>)	<i>Viola tricolor</i>
<i>Syringa vulgaris</i>	<i>Umbellularia californica</i>	<i>Vitis</i> sp.
<i>Tamaria</i> sp.	<i>Ursinia</i> sp. (<i>Sphenogyne</i> sp.)	<i>Withania somnifera</i>
<i>Taraxacum officinale</i>	<i>Urtica pilulifera</i>	<i>Zea mays</i>
<i>Thalictrum minus</i>	<i>Urtica urens</i>	<i>Zelkova formosana</i> [sic]
<i>Tilia americana</i>	<i>Valeriana pyrenaica</i>	
<i>Townsendia exscapa</i>		
(<i>T. sericae</i>)		

Distribution. The green peach aphid appears to be present throughout the entire world wherever agricultural crops are grown. It does not occur in the extreme cold areas of the Arctic Region nor in certain of the very hot desert oases. Whether this wide distribution is natural or aided by man cannot now be determined. Nevertheless man has indeed had a great influence in extending both its distribution and diet by the extensive development of agriculture in areas which were previously certainly not adapted to the requirements of this aphid.

In California it occurs in every county and is a pest on plants in houses, greenhouses, lathhouses, and out doors throughout the year in most localities.

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ANATOMIC EFFECTS OF THE VIRUSES OF PIERCE'S DISEASE AND PHONY PEACH¹

Katherine Esau²

INTRODUCTION

PREVIOUS INVESTIGATIONS have shown that in its method of transmission Pierce's disease virus is closely associated with the xylem of the hosts (Houston *et al.*, 1947).³ Furthermore, the external symptoms of the diseases caused by this virus—Pierce's disease of the grape and dwarf disease of alfalfa—suggest a disturbance in the water-conducting system (Hewitt *et al.*, 1942b; Weimer, 1931). At present no other virus is known to be so definitely associated with the xylem as that of Pierce's disease. A study of the pathological anatomy of the hosts of this virus is therefore of particular interest in the problem of tissue relations of viruses. The investigation reported in this study was undertaken to determine whether the anatomic symptoms occur in the xylem and to what extent, if at all, they are restricted to this tissue. An attempt was also made to determine the nature and location of the first signs of histopathological disturbances. This was done through developmental studies on infected grape seedlings and cuttings of alfalfa.

Certain viruses cause degenerative changes in the xylem, without necessarily being limited to this tissue. Among these, the psorosis virus is particularly well known. The anatomy of the affected wood of citrus has been described (Fawcett and Bitancourt, 1943; Webber and Fawcett, 1935). Phony peach virus also appears to cause disturbances in the xylem (Hutchins, 1933), but the information concerning its anatomic effects on this tissue is not available. The pathological anatomy of phony peach roots has been briefly considered in the present study in order to obtain additional information on the effect of viruses on the xylem.

REVIEW OF LITERATURE

Pierce's disease of the grapevine was recognized first as a graft transmissible virus disease (Hewitt, 1939); then several of its insect vectors were discov-

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³ See "Literature Cited" for complete data on citations, referred to in the text by author and date of publication.

ered (Hewitt *et al.*, 1942a). The identification of alfalfa dwarf as a virus disease transmitted through root grafting was made independently of the studies on Pierce's disease of the grapevine (Weimer, 1936). Later, alfalfa dwarf and Pierce's disease were found to be caused by the same virus and transmitted to the two hosts by the same insect vectors (Hewitt and Houston, 1941; Houston *et al.*, 1942; Hewitt *et al.*, 1946).

The external symptoms of Pierce's disease of the grapevine, particularly on *Vitis vinifera*, have been considered in detail by Hewitt *et al.*, (1942b). Briefly, these symptoms are as follows. Delayed growth in the spring; mottling and, later, burning and scalding of leaves; wilting and drying of fruits; dwarfing of parts or of entire vines; and uneven maturation of canes. Sudden wilting is characteristic of young, vigorously growing diseased vines, and a drying of many leaves or parts of leaves follows this wilting. Young and old vines die from the disease, sometimes in the first season of infection or one or more years later.

According to Weimer (1931) and Weimer and Madson (1932), alfalfa plants affected by the dwarf disease are, as the name implies, dwarfed in appearance, because they have short stems and rather small leaves. After each cutting, the stems become shorter and more slender and the leaves smaller. The number of stems also diminishes. Often the plants are an unusually dark green and, in the late stages of the disease, the tips of the stems sometimes wilt even in wet soil. There is, however, no mottling or crinkling or any other pronounced deformation of leaves. Blossoming is often delayed or completely inhibited.

Pierce's disease virus occupies a unique place among the known plant viruses. Whereas viruses thus far have been divided into those that are more or less localized in the phloem or in the parenchyma outside the phloem and those that are not limited to any tissue and occur in both the phloem and the parenchyma (Bennett, 1940), Pierce's disease virus is closely associated with the xylem. This relation has been clearly revealed by observations on the mode of vector feeding and on the tissues involved in the transmission of the virus (Houston *et al.*, 1947).

All the insect vectors of Pierce's disease virus, whether kept on the grapevine or on alfalfa, seek out the xylem in feeding, and the virus can multiply and cause the disease only when the xylem tissue is actually reached by the vector. Experiments on alfalfa have shown that occasionally the virus moves upward quite rapidly. This observation and the information that insects puncture the walls of the tracheary elements in feeding on the xylem (Houston *et al.*, 1947) suggest that the virus can move with the water stream in the water-conducting elements of the xylem.

In agreement with the conclusion just mentioned, some of the external and internal symptoms of the disease in the grape and alfalfa indicate that xylem is the tissue primarily affected by the disease. The sudden wilting of vigorously growing young vines and the scalding and drying of leaves in the grapevine, the wilting of stem tips and the dark coloration of the foliage in alfalfa point toward insufficient delivery of water to the tops of the plants. According to the early histologic studies on Pierce's disease, the wood of affected grapevines is discolored and gum and tyloses occur in the tracheary

elements (Dowlen, 1890; Viala, 1893; Butler, 1910). In dwarf alfalfa, also, the vessels are plugged with gum (Weimer, 1931, 1936).

For comparison with the results of the present study, the references dealing with the histology of plants affected with Pierce's disease virus are here reviewed in some detail. Dowlen (1890) appears to have been the first to report on the discoloration of the wood of affected grapevines, the filling of cell cavities with black-brown deposit, and the development of tyloses—sometimes very abundant—in the vessels. He also found moisture and starch to be deficient in the canes that failed to ripen, or in the immature areas of canes that ripened unevenly. The small amount of starch present in unripened areas did not readily turn blue on application of iodine, the individual grains were small and seemingly partly eroded. Dowlen compared Pierce's disease with mal-nero of Sicily and Italy, which also causes starch deficiency in stems and the appearance of brown matter and of tyloses in the vessels.

Butler (1910) has explained that the retention of the green color in the immature areas of the cane results from nondevelopment of a cork layer that normally cuts off the chlorophyll-containing cortex in a maturing cane of a healthy plant (see Esau, 1948*a*). In the mature areas cork is formed, however. Butler also observed starch deficiency in immature canes, but was in error when he assumed that the uneven thickness of the vascular tissues in the different parts of the circumference of a cane—a feature characteristic of grape canes (see Esau, 1948*a*)—resulted from the presence of the disease. Butler mentioned tyloses in primary and secondary wood, and gumlike deposits in cells of all tissues of the cane, but the main object of his histologic study was the mottled leaf.

According to Butler (1910), the degenerative changes vary in different parts of the affected leaves. In the chlorotic areas, chloroplasts degenerate. They become less discrete and aggregate into plasmodia-like masses, which finally disappear. Such degeneration is particularly characteristic of the palisade tissue. In the spongy layers plastids may become vacuolate, but do not distend, and either fragment or degenerate into oil-like bodies. The parts of leaves showing red discoloration, on the other hand, have an overabundance of starch in the chloroplasts. When the reddened areas die, starch disappears, and the cells become filled with a morphologically variable occluding matter. Different amounts of this substance occur also in cells that are not dead. Microchemical reactions of the occluding matter suggested to Butler that the latter was composed of tannin, proteinaceous materials, and wound gum. The absence of occluding matter in the chlorotic areas where the plastids degenerated completely, and its presence in parts where starch was plentiful before the appearance of this matter, indicated to Butler that the occluding substance was derived mainly from decomposed starch. Various degrees of occlusion of vessels by the gumlike substance or by tyloses also occurred in the leaves.

Butler (1910) found no symptoms of diagnostic value in the arms, trunks, and roots of the grapevine, and concluded that Pierce's disease primarily affected the leaves, fruit, shoots, and canes.

The only symptom of alfalfa dwarf observed in the roots was the deposition of gum in the vessels (Weimer, 1931, 1936; Weimer and Madson, 1932).

According to Weimer (1931), in the earliest stage of disease development the wood becomes yellow just beneath the bark. This yellowing, which results from the appearance of gum in the vessels, spreads from the outer portion of the root toward the interior. Frequently, when the plant dies, the root is discolored throughout. During the winter and early spring, the discoloration does not extend to the cambium but is separated from it by a layer of new wood. Weimer thinks that this distribution of the symptom indicates inactivity of the disease during the winter months. The yellow discoloration occurs also in the main divisions of the crown and in the bases of the green stems, but apparently does not extend far up into the latter.

The gum accumulation in vessels, although characteristic of plants infected with Pierce's disease virus, is not a specific symptom of this malady. It may be caused in alfalfa and the grapevine (as well as in other plants) by other pathogens and certain physiological disturbances. The bacterium *Aplanobacter insidiosum* L.Mc., the causal agent of alfalfa wilt, induces symptoms in alfalfa roots, which are very similar to symptoms in plants affected with alfalfa dwarf. The invasion of the vessels by the bacteria is followed by deposition of gum in these elements (Jones and McCulloch, 1926). Later, the gumlike material appears also in vessels beyond those invaded by the bacteria (Jones, 1928*b*). The deposition of gum is accompanied by a change in the composition of the walls of groups of cells adjoining the vessels, as is evidenced by their brilliant staining with phloroglucinol and hydrochloric acid (LeClerc and Durrell, 1928).

A similar plugging of vessels in alfalfa roots may be induced by a high water content in the soil and by injections of various salt solutions (LeClerc and Durrell, 1928). Jones (1928*a*) found gum formation in the vessels of alfalfa after winter injury to the roots and after injection of dilute acids through the cut ends of the stem. Alfalfa roots affected with the wilt disease are low in starch. The depletion of the latter is obviously related to the plugging of vessels: the starch disappears first of all near these vessels (Peltier and Schroeder, 1932). Tyloses appear to be rare in alfalfa roots (Jones, 1928*a*; LeClerc and Durrell, 1928).

The grapevine also produces gum and tyloses in the xylem under varied conditions, some of which may not be abnormal (Mangin, 1895; Rathay, 1896). Rathay studied gum formation in grapes in particular detail, and used more than one species of *Vitis* and even other representatives of the *Vitaceae*. He found that in all species of *Vitis* and in all cultivated varieties which he had studied, the two- to several-year-old growth of the aboveground parts contained some vessels with gum. In one variety of *Vitis vinifera* he found gum in one-year-old wood also. Gum development in roots appeared to be less common, less regular, and later than in the stem parts. The sapwood, according to Rathay, is transformed into heartwood between its twentieth to thirtieth years. Gum formation is not associated with this change. In fact, as the heartwood is formed, tyloses develop in vessels containing gum.

Rathay also studied gum formation caused by wounding. Below transverse cuts of young shoots (one to two years old) the tissues died part way. Beneath the dry dead part, vessels became clogged with gum and, still lower, tyloses developed in the same vessels that contained gum at the higher level.

Some workers have given considerable attention to the nature and origin of gum in plants. The grapevine gum, according to Ráthay (1896), gives the reaction of pentoses, has a strong swelling capacity, and contains over 50 per cent of water. Ráthay was of the opinion that gum was formed in the xylem-parenchyma cells, but that it probably left these cells as a slowly diffusing colloid and only then assumed the nature of gum. He decided that the gum occurred under pressure in the vessels—hence, its exudation from cut branches—and this pressure did not change with the seasons.

According to Tschirch (1889), plant gum is derived from decomposition of cell walls, but is also apparently produced by living cells. Butler (1911) strongly emphasized that cell contents, including the starch, were in no way concerned with gum formation in *Prunus* and *Citrus*: the gum resulted solely from the hydrolysis of walls. (In the grape, however—Butler, 1910—as stated previously, associated the gummy deposit in the mesophyll with starch decomposition.)

Bartholomew (1928), on the other hand, considered that in endoxerosis of lemons both starch and cell wall were the sources of gum. He described a swelling of the middle lamella, in tissues other than the xylem, in the early stages of gummous degeneration, then a breakdown of the rest of the wall. When gum developed in the xylem, the middle lamellae of the walls between the parenchyma cells and vessels were affected first, then the cellulose wall, and finally the starch in the parenchyma cells. The walls, however, were not completely destroyed. Bartholomew observed accumulations of gum in the pit cavities on the parenchyma side. This gum then passed through the pits, and appeared as droplets on the inner face of the vessel wall. The droplets later coalesced and filled the vessels. The gum had variable properties during the different stages of its development. At first it was water soluble, and remained thus for at least a week or 10 days after it migrated into the vessels. Later it became less soluble and finally entirely insoluble in water. Bartholomew suggested that perhaps only at this last stage should the substance be referred to as "gum." Since the processes of conversion of the pectic substances, cellulose, and starch into water soluble or insoluble gums are highly complex, many intermediate substances are formed before the endproduct is reached. This complexity explains the variability in the staining reactions and properties of the gum at the different stages of its development.

Peltier and Schroeder (1932) suggested that the gum in alfalfa roots affected with wilt might be the residue (possibly lignin) left after the bacteria have dissolved out the soluble pectins from the middle lamella.

The virus disease of citrus called "psorosis" (Fawcett and Bitancourt, 1943; Bitancourt *et al.*, 1943) induces, among other symptoms, gum deposition in the vessels, which is associated with starch depletion in the surrounding cells. The authors, therefore, assume that the gum is derived from the starch.

A summing up of the literature on gummosis just reviewed indicates that gum deposition and tylose development in the tracheary elements of the xylem are frequent phenomena. They may occur in the xylem of various plants under apparently normal conditions, but particularly when the tissue is affected by various pathogens and physiological disturbances. Most workers agree that the gum is derived from decomposition of carbohydrates—notably

starch—and that the products of decomposition migrate from the living cells into the tracheary elements. Most often, the gum has been identified—in its final state of transformation—as a wound gum giving lignin reaction.

Phony disease of peach causes a dwarfing of branches, twigs, and fruit of the affected tree. With each succeeding year the amount and quality of the fruit are reduced. The disease, however, does not kill infected trees, nor does it cause any yellowing of the leaves. On the contrary, the foliage of the diseased trees appears more vigorous than that of the healthy (Hutchins, 1933).

The only known internal symptom of the disease is the appearance of well-distributed purplish spots in the wood of fresh roots treated with acidulated absolute methyl alcohol (Hutchins, 1933). Since Hutchins (1933) was able to transmit the virus by grafting of roots but not of tops, he concluded that the virus did not invade the branches of the tree. Moreover, Hutchins (1939) obtained evidence suggesting that the virus was localized in the woody cylinder of the root: the virus was transmitted if whole root sections were used in grafting, but not if the sections contained only the bark.

MATERIAL AND METHODS

The grapevine (*Vitis vinifera* L.) and alfalfa (*Medicago sativa* L.) material was obtained partly from plants grown and inoculated with Pierce's disease virus in a greenhouse at Davis, and partly from field-grown plants naturally infected with the virus in various localities of California. Healthy material was available in the same locations as the diseased. The inoculations in the greenhouse were made by using the insect vectors of the virus. The healthy and phony-diseased peach samples were imported in preserved condition from Brownwood, Texas.

The younger and softer material was processed by an ordinary paraffin method, with tertiary butyl alcohol used as a dehydrating agent. The older branches and roots were sectioned—fresh or after preservation in standard killing fluids—on sliding or freezing microtomes. In general, the methods followed were those previously described in detail (Esau, 1948a) and much of the material was stained with Bismarck brown and iodine green and mounted in Karo syrup.

PATHOLOGICAL ANATOMY OF THE GRAPEVINE AFFECTED WITH PIERCE'S DISEASE

Structure of Vegetative Organs of Healthy Vines. The structure of the axis of various ages of healthy grapevines is described in an earlier paper (Esau, 1948a). It is briefly outlined here.

At the end of primary growth a seedling stem or a shoot on an old vine have, from inside out, a parenchymatous pith, xylem, cambium, phloem, cortex, and epidermis. The xylem and phloem are arranged in the form of collateral strands separated from each other by wide parenchyma rays. Through secondary growth, new vascular tissues are formed, and the epidermis, cortex, and some phloem are cut off by a cork cambium originating in the phloem (plate 8, B). The interfascicular cambium formed in the rays

produces ray parenchyma. From time to time some fascicular cambial initials are converted into ray initials and, thus, rays arise within the vascular strands. All the rays are wide, so that the secondary vascular region, like the primary, is conspicuously broken up into radial blocks by parenchyma cells (plate 6). Since new cork cambia arise year after year in successively deeper layers of the phloem, the latter tissue is periodically sloughed off, and only a relatively small amount of nonfunctioning phloem accumulates in the bark of the axis. When, at the end of the season, cork is formed in a current-year shoot, the latter becomes the "mature cane." Several-year-old branches bearing the cane are called "arms" by viticulturists. The roots are without pith but have rays, many of which are wider than the rays of stems. The cork cambium cuts off some phloem in the root periodically, but less regularly than in the stem.

The secondary phloem is composed of two kinds of cell masses occurring in alternate radial blocks: the rays (transverse system) and the longitudinal system. The latter consists of alternate tangential tissue bands: the sieve-tube bands and the fiber bands (plate 6, *B*, to the left in the photograph; the fiber bands are narrow and dense). The sieve-tube bands contain sieve tubes, companion cells, and phloem parenchyma. Most of the fibers are septate, and all remain alive until they are separated from the stem by cork. Starch storage occurs in phloem parenchyma, ray parenchyma, and fibers.

The secondary xylem also consists of radially alternating blocks of the transverse (rays) and longitudinal systems (plate 6, *A*). The latter is composed of vessels, fibers, and xylem parenchyma. (According to Penzig, 1882, tracheids also occur in *Vitis*.) The largest structures visible in a section of xylem are vessels (plate 6, *A*), but some of these elements are very narrow. Vessels occur singly or in small groups. The superposed vessel members are connected by simple circular or elliptic openings, sometimes by scalariform perforation plates (Penzig, 1882; Ráthay, 1896; Solereder, 1908). The pit pairs between two adjacent vessels are bordered and elongated. Half-bordered pit pairs occur between vessels and xylem parenchyma cells (plate 10, *A*). The narrow thick-walled cells among the vessels are fibers. These are mostly septate, and contain a nucleus in each compartment. Their pits are bordered and have slitlike openings. The xylem-parenchyma cells surround the vessels and are also scattered among the fibers. The rays consist of parenchyma cells only. Many xylem- and ray- parenchyma cells show dark contents because of the presence of tannins (plate 6, *A*). Starch storage occurs in the xylem parenchyma, ray parenchyma, and fibers. Some of the vessels become plugged with tyloses at the end of the season and these structures also store starch. Tyloses are formed by xylem-parenchyma cells and eventually develop thick, pitted walls.

The anatomy of the grape leaf was described by Penzig (1882) and Solereder (1908), and the present observations agree with the reports of these workers. A single-layer epidermis covers the leaf on both sides and the stomata are restricted to the lower epidermis (plate 1, *A*). The leaf is prominently bifacial, having one layer of very long palisade cells (sometimes two layers) and several layers of spongy parenchyma (plates 1, *A*; and 2, *A*).

The palisade cells have rather dense contents (some of which are probably

tannins) which obscure the chloroplasts. The spongy-parenchyma cells also contain darkly staining material, which is even denser than that of the palisade cells. The chloroplasts in the spongy parenchyma are smaller and apparently fewer in number than in the palisade layer.

The spongy-parenchyma cells next to the palisade differ from the other cells of the same layer: the tannin in these cells occurs in the form of small droplets, and the plastids, although relatively small, are abundant and conspicuous (plates 1, A; and 2, A, at *a*). Some cells within the spongy parenchyma enlarge greatly and become the containers for raphides, the "raphide sacs." The protoplasts of these cells become disorganized, and the crystals remain imbedded in a mucilaginous substance. Other types of crystals also occur in the mesophyll, but their development is not associated with unusual cell enlargement.

The chloroplasts are usually conspicuously flattened, and sometimes cover the palisade wall with an almost uninterrupted layer. They may contain small starch grains (the light dots in some plastids in plate 2, A).

The vascular system of a leaf consists of 5 major veins and numerous anastomosing ones of various sizes. Among the major veins the median is the most robust. The large veins have several bundles each (arranged in a circle in the largest veins) imbedded in vein parenchyma. The net of vascular bundles anastomosing in the mesophyll between the large bundles is located in the upper part of the spongy parenchyma (plate 1, A). Each bundle is surrounded by a sheath of border-parenchyma cells with very small chloroplasts and usually with almost no tannin (plates 1, A, and 3, A). These parenchyma cells are elongated parallel to the bundle course (plate 3, A). The somewhat larger bundles traversing the mesophyll are not only imbedded in a sheath of parenchyma cells, but are also connected with the epidermis by means of similar cells (plate 1, A, at *c*). These connecting cells constitute the vein extensions considered in such detail by Wylie (1943).

Internal Symptoms of Pierce's Disease. The present observations on the symptoms of Pierce's disease generally agree with those of other workers (see the review of literature), except that, contrary to Butler's report, the anatomic effects were found in axes of various ages and not in the one-year-old canes only. In the following paragraphs is a general description of the internal symptoms, whereas the details on the distribution of these symptoms in different parts of the vine and on their development appear in the succeeding parts of this paper.

Anatomic changes induced by Pierce's disease occur in the xylem, the bark, and the mesophyll. Two types of development occur in the xylem: gum deposition in various xylem cells, including the vessels, and occlusion of vessels by tyloses. According to the literature reviewed previously, gum and tyloses may be induced by various diseases and by physiological disturbances, and tyloses are common in normal plants, but the prevalence of these formations in grapes affected with Pierce's disease (see next part of this paper), justifies their classification as symptoms of the disease. These symptoms may be found in the primary and secondary wood in vegetative parts of various kinds and ages. (Floral parts were not included in this study.)

Several photomicrographs illustrate the effect of Pierce's disease upon the

xylem. Plate 4 compares vascular bundles from petioles of a healthy (*A*) and of a diseased (*B*) vine. In the former the younger vessels are all intact, and their lumina are free of any obstructions. The older vessels (protoxylem) have been partly crushed by xylem parenchyma in the process of normal obliteration (plate 4, *A*, at *a*). The bundle from the diseased vine shows gum in several vessels (plate 4, *B*, at *b*). Obliteration of the protoxylem vessels in this bundle has occurred in a normal manner. In addition, some xylem-parenchyma cells in the oldest part of the bundle have undergone several divisions (plate 4, *B*, at *c*).

Such renewal of meristematic activity was encountered only occasionally in the present study, but evidence was obtained that it resulted sometimes in a differentiation of complete vascular bundles with xylem and phloem. In the bundle in plate 11, *E*, such supernumerary bundles appear at *c*. In the median of the three bundles the tracheary elements occur next to the radial row of old vessels, which appears above in the picture, and the sieve tubes are next to the median of the three radial files of vessels.

Gum deposition may occur in all types of cells in the xylem. Plate 7, *B*, shows heavy accumulations of gum in some narrow vessels, many fibers, and some ray cells (below the wide vessels). In this section, gum was evident in the xylem parenchyma, also, but this phenomenon is more clearly illustrated in plate 14, *B*, where the parenchyma cells surrounding the vessel are partly filled with gum. The vessel in plate 14, *B*, contains both gum and tyloses. The latter are responsible for the partitioning of the vessel lumen.

The gum is yellowish brown in fresh sections and, when treated with phloroglucinol and hydrochloric acid, shows different tones of yellow, orange, and red. The latter color is somewhat more brilliant than the red of the lignified walls, probably because of the admixture of yellow in the red coloration of the gum. In sections stained with safranin and fast green, the gum stains in various tones of green, greenish yellow, and red, and mixtures of the three. As with phloroglucinol and hydrochloric acid, the red coloration of the gum obtained with safranin is more nearly scarlet, whereas that of the lignified walls is purplish red. Bismarck brown and iodine green stain the gum a brownish purple (plate 14).

As Bartholomew (1928) has suggested, the variability in staining reactions of the gumlike deposits is probably a reflection of the variability in properties of the substances intermediate between the carbohydrates and the end product of their decomposition—the gum proper. When the latter is present, red coloration is obtained with the phloroglucinol-hydrochloric acid combination and with safranin. At this stage the walls of vessels containing the gum often stain like the gum itself, particularly the pit-closing membranes. The latter, moreover, become more or less swollen. In plate 14, *A*, the two pit-closing membranes in the middle of the figure, extending along the entire sides of the vessels (evidently the pits are much elongated), are thick and are stained a purplish brown, whereas elsewhere the primary walls are thin and light yellow in color. (The vessels in this figure have gum and tyloses. The walls of the latter are closely appressed to the walls of the vessel, except where gum occurs between the tylose and the vessel wall.)

Tyloses occur in vessels of all sizes, and may or may not be associated with

gum in a given section (plates 6, *B*; 7, *A*; and 10, *F*). In longitudinal sections, the tyloses are seen occluding the vessels for long distances (plate 9, *B*). Similarly the gum, where present, fills few or many of the successive members of one vessel.

The development of tyloses was studied in some detail. To all appearances these structures are formed through an enlargement of the pit-closing membranes of the pit pairs located between the xylem-parenchyma cells and the vessels. Unmodified pit membranes are shown in plate 10, *A*, whereas those in plate 10, *B*, and *C*, more or less protrude into the vessel lumina. The membrane appears fairly thick in the first stages of growth (plate 10, *C*), but later becomes thinner (plate 10, *D*).

The nuclei migrate from the parenchyma cells into the tyloses (plate 10, *D*). Often more than one tylose arise from the same parenchyma cell (plate 10, *E*). The behavior of the nucleus in such instances is not clear. As far as observed, the nucleus occurred in only one, the largest of the several tyloses from a given cell. In plate 10, *E*, for example, only the larger of the two tyloses showed a nucleus (its shadow is visible in the photograph). Perhaps the enucleate tyloses do not grow very long—a suggestion supported by the observation that, in thoroughly plugged vessels, small tyloses occur at the bases of the large ones, which reach far into the vessel lumen.

Tyloses obviously arise from many of the cells lining the periphery of a given vessel, and tannin-free and tannin-containing cells take part in this growth (plate 10, *F*). Tyloses arising from the different parts of the vessel circumference eventually touch one another and thus occlude the vessel lumen completely (plates 7, *A*, to the left, and 9, *B*). After they cease to grow, tyloses develop thick walls with simple pits and may become lignified.

The effect of Pierce's disease upon the bark of the cane is associated with the imperfect maturation of the latter. As was mentioned previously, a cane on a healthy vine entering dormancy stores starch in all vascular tissues, and forms a cork cambium inside of the primary phloem fibers (plate 8, *B*). In diseased canes, starch storage is incomplete and cork cambium fails to develop in more or less extended areas (plate 8, *A*).

Since cork formation causes death and browning of tissues outside of the cork, the patches of cane periphery having none of the latter retain live, green cortex and fail to assume the brownish-grey color characteristic of the bark of mature canes (Hewitt *et al.*, 1942). The uneven formation of cork may be observed in old axes also. Plate 9, *A*, shows a section of a cane in the second year of growth with new cork (at *b*) formed only partially and united with the old cork layer at *a*. The phloem itself shows no histologic abnormalities, except that the irregularity in cork formation may cause an accumulation of excessive amounts of nonfunctioning phloem. In severely affected plants there may be a suppression of phloem development, along with that of the xylem.

The leaves from diseased vines show variable degrees of degeneration. The younger leaves appear to be free of abnormalities as long as they show no mottling. The older leaves exhibit the various features described by Butler (1910). Some bundles show occlusion of vessels with gum (plate 1, *B*, at *d*). One or two or several vessels contain gum, or all are occluded. The mesophyll cells develop variable amounts of material that takes up stains readily. This

material, which, according to Butler (1910), contains gum, tannin, and other substances may emerge into the intercellular spaces (plate 1, *B*; note gum in substomatal cavities above *b*). The severely affected (yellow) leaf areas are thin, and appear incompletely differentiated because the intercellular-space system is poorly developed. If the latter is filled with the gumlike material, the thin dense mesophyll differs strikingly from the thick lacunose tissue of the healthy leaves. (Compare *A* and *B* in plate 1.)

The plastids of diseased leaves tend to accumulate abnormal amounts of starch, particularly in the yellow areas. Plate 2, *B*, shows chloroplasts with starch in the palisade and spongy parenchyma. In plate 3, *B*, the starch grains in the border parenchyma of a bundle are particularly large and uneven in shape and size. In leaves stained with iodine the deepest blue appears in the border parenchyma, as though the carbohydrates were dammed off here. The chloroplasts of the yellow areas lack the normal amounts of chlorophyll. In some parts of the mesophyll the plastids degenerate more or less thoroughly. They fuse into amorphous masses, as in beet leaves affected with the mosaic (Esau, 1944), and eventually break down completely. Sometimes entire cells or cell complexes undergo necrosis. The latter symptom is expressed externally as leaf scalding (Hewitt *et al.*, 1942*b*).

The abnormalities just described are irregularly distributed in a given leaf, and the yellow areas show more pronounced degenerative changes than the green. The chloroplasts in the latter develop smaller starch grains, and the gum is less common than in the yellow areas. Thus, there is a quantitative difference between the green and the yellow areas in degenerative changes, but both are abnormal.

Effect of the Disease on Secondary Vascular Tissues in Axes of Various Ages. According to the review of literature, the principal effect of Pierce's disease was to be sought in the xylem, but the irregularities in cork formation suggested that the phloem also might be affected by the presence of the virus in the plant. To obtain some preliminary information on the condition of the phloem in diseased vines, bark samples from eight diseased and five healthy (that is, free from symptoms*) vines were collected from a vineyard in Napa Valley on June 14, 1945. The material was sectioned fresh, and was treated with iodine and aniline blue.

The phloem of the diseased and the healthy vines was compared for the number of annual increments present in the bark, the composition of these increments (that is, number of fiber and sieve-tube bands), the thickness of the whole phloem and of its functioning part, and the per cent of functioning phloem (table 1). In addition, the phloem of affected plants was examined for any evidence of specific degenerative changes. No such changes, however, occurred in this set of samples (nor in any other collected for the present study). The features identifying the nonfunctioning part of the phloem were the same in the healthy and in the diseased vines: partial collapse of sieve tubes and presence, in these elements, of residual material, tyloses, and definitive callus; the absence of slime and starch in the cell lumina and of connecting strands in the sieve plates. (See Esau, 1948*a*.)

* In field collections there was, of course, no assurance that vines free from symptoms were also free of Pierce's disease virus. Nevertheless, for convenience of expression the check plants are here spoken of as "healthy plants."

On June 14, when the collection was made, radial growth had not yet ceased, and no 1945 cork was present in the samples. The bark contained phloem formed in the past seasons and some 1945 phloem. In most samples, the old phloem was represented by the 1944 increment. Two of the diseased and one of the healthy vine samples contained the 1944 and the 1943 phloem incre-

TABLE 1
CHARACTERISTICS OF THE PHLOEM OF GRAPEVINES WITH AND WITHOUT
SYMPTOMS OF PIERCE'S DISEASE

Vine number	Condition of vine	Rating of disease*	Number		Thickness in mm		Per cent of functioning phloem
			Of complete annual increments	Of fiber bands in complete increments†	Of total phloem	Of functioning phloem	
Carignane							
1.....	Diseased	4	1	2	0.67	0.25	37.3
2.....	Diseased	4	1	3	0.83	0.70	84.3
3.....	Diseased	3	1	3	0.82	0.65	79.3
4.....	Diseased	2	1	4	1.35	0.70	51.9
5.....	Healthy	0	1	4	1.47	1.27	86.4
6.....	Healthy	0	1	3	1.32	1.00	75.8
Green Hungarian							
7.....	Diseased	2	1	4	0.93	0.65	69.9
8.....	Healthy	0	2	4	1.02	0.75	73.5
Palomino							
9.....	Diseased	4	1	4	1.00	0.72	72.0
10.....	Diseased	4	1	4	1.28	0.62	48.4
11.....	Diseased	3	1-2	4	1.07	0.79	73.8
12.....	Healthy	0	3	6	1.38	0.70	50.7
13.....	Healthy	0	1	4	1.18	0.87	73.7
Palomino, 4 separate samples of vine 11							
11.....	Diseased	3	1	4	1.05	0.75	71.4
11.....	Diseased	3	1	4	1.15	0.88	76.5
11.....	Diseased	3	2	4	0.83	0.57	68.7
11.....	Diseased	3	1	4	1.25	0.95	76.0
Average of diseased....		..	1.0	3.5	0.99	0.64	64.6
Average of healthy....		..	1.8	4.0	1.25	0.92	73.6

* Rating by Dr. Wm. B. Hewitt. 0, no symptoms; 4, most severe symptoms.

† The number of sieve-tube bands is one, or a fraction of one, higher than the number of fiber bands. (See Esau, 1948a.)

ments, and one of the healthy plants had three old increments in the bark. (Item "Complete annual increments" in table 1.) The number of fiber and sieve-tube bands also varied, mainly in relation to the total thickness of the phloem. Similar variations in the number of annual increments and in the number of fiber and sieve-tube bands were observed in the bark of trunks of the healthy *Vitis vinifera* considered in a previous study (Esau, 1948a).

The old phloem and the 1945 phloem together gave the values for the total phloem thickness in table 1. More or less of the old phloem was in functioning state, since this tissue was partly reactivated in the early part of the growth season (Esau, 1948a). The reactivated phloem and the new (1945) phloem

were measured together for the value of thickness of functioning phloem in table 1. Although the amount of the 1945 phloem differed from sample to sample, the thickness of the reactivated phloem varied even more. This latter variation largely determined the fluctuations in the per cent of functioning phloem.

In the material used for table 1 the phloem from healthy vines was, on the average, somewhat thicker than that from the diseased, but individually some diseased vines showed thicker phloem than the healthy. Moreover, the figures for diseased vine 11 indicate that a given plant may show variations in bark thickness in different parts of the trunk similar to those between samples from healthy and diseased vines. (See Esau, 1948a.)

The per cent of functioning phloem in the healthy vines was, on the average, higher than in the diseased. A sample-by-sample comparison, however, shows no constancy in this difference. The sample from the healthy vine 12, for example, had almost as low a per cent of functioning phloem as those from diseased vines with the lowest values for this item.

The variations in the per cent of functioning phloem could result either from irregularities in cork formation, and the consequent excessive accumulation of old nonfunctioning phloem, or from the failure of rather large amounts of the old phloem to become reactivated. In vine 1 (table 1) the low per cent of functioning phloem was caused by a nonoccurrence of reactivation in most of the old phloem: the functioning phloem formed a small part of the rather thin total phloem. The other three vines with uncommonly low per cent of functioning phloem,—two diseased (vines 4 and 10) and one healthy (vine 12)—had very thick total phloem but only a moderate amount of functioning phloem. In these vines the low per cent of the latter tissue resulted from excessive accumulation of old phloem. Probably both phenomena, incomplete reactivation and imperfect cork formation, play a role in producing barks with excessively low per cent of functioning phloem.

The data in table 1 show no correlation between the condition of the phloem and the severity of symptoms on the vine. In the Carignane variety the more severely affected vines had comparatively thin phloem, but the per cent of functioning phloem was not related to the symptom rating. In the Palomino, one of the severely affected vines had a very low, the others a moderate per cent of functioning phloem.

Thus, this preliminary study showed no specific nor constant quantitative differences between healthy and diseased vines with regard to the structure of the phloem, but it indicated that the total and relative amounts of the functioning phloem may be reduced by the disease.

Two subsequent series of studies were designed to determine the condition of both the xylem and the phloem in the diseased vines as compared with the healthy. The first of these two series of studies concerned two- to five-year-old arms of diseased and of symptom-free vines of two field-grown varieties—Emperor and Palomino. The Emperor was growing in the San Joaquin Valley, and, since the vineyard was rogued each season, the available diseased vines were mostly those developing the leaf symptoms during the season of sampling, and were only moderately affected. The Palomino vines were growing in the Napa Valley. They were affected by the disease for variable lengths

TABLE 2
XYLEM AND PHLOEM CHARACTERISTICS IN ARMS FROM EMPEROR AND PALOMINO GRAPEVINES
WITH AND WITHOUT SYMPTOMS OF PIERCE'S DISEASE

Variety	Symptoms present or absent	Age of arm in years	Number of samples	Thickness in mm of annual rings of xylem in the various years			Thickness in mm of phloem		Per cent of functioning phloem	Per cent of vessels with tyloses in the xylem of the various years		
				1946	1945	1944	Total	Functioning		1946	1945	1944
Emperor	Absent	2 to 4	31	1 90	2 12	2 66	0 70	0 55	78 65	5 77	9 13	14 24
Emperor	Absent	3	28	1 85	2 11	2 78
Emperor	Present	2 to 5	31	1 75	1 82	2 37	0 65	0 46	70 33	57 30	53 42	41 53
Emperor	Present	3	18	1 84	2 13	2 87
Palomino	Absent	3 to 5	31	1 30	1 72	2 11	0 51	0 39	77 14	12 41	22 09	29 13
Palomino	Absent	3	27	1 32	1 86	2 37
Palomino	Present	3 to 5	31	1 50	1 80	2 21	0 56	0 40	70 84	44 17	42 90	36 89
Palomino	Present	3	26	1 59	1 92	2 42

of time, and consequently showed symptoms of different degrees of severity. The water conditions in the Napa Valley vineyard were less favorable than those in the San Joaquin Valley vineyard.

The collections were made September 18, 1946, from the Emperor variety, and November 2, 1946, from the Palomino variety. In each variety, 31 diseased and 31 healthy vines were sampled. The plants without symptoms were scattered among those showing symptoms, and the two kinds of samples were taken in pairs from vines growing as close as possible to each other. An effort was made to select arms of the same age, preferably those in their third season of growth. The samples, however, proved to vary in age, from two to five years in the Emperor and from three to five years in the Palomino.

The data obtained from these samples were assembled in table 2. The following three kinds of characteristics were determined: thickness of annual rings of xylem formed in each of the three years given in the table; thickness of phloem and per cent of functioning phloem in the total bark thickness; per cent of vessels with tyloses in each of the three latest annual increments.

Each sample listed in the fourth column of table 2 consisted of one half of a circumference of one arm. The thickness of xylem and phloem was measured in five places distributed as evenly as possible through each increment. (The phloem had usually only one complete increment and a small part of another, which was measured together with the complete increment.)

The count of tyloses was made in ten different portions of each xylem increment. Usually all vessels (except the smallest, which were difficult to recognize at the low magnification used) of a block of tissue enclosed between two rays were counted. The total number of vessels in the ten counts ranged from about 250 to over 400. Gum formation was frequently associated with the tyloses. It occurred in cells around the vessels and also in the lumina of the latter. Sometimes the vessels were filled with gum but contained no tyloses. Such vessels were included in the count together with those filled with tyloses.

Table 2 presents the various averages for each lot of 31 samples and also the averages of xylem thickness for the three-year-old arms selected out of each group of 31 samples. Such treatment seemed necessary because usually the first annual increment of a given arm was somewhat wider than the subsequent ones, and the lots that contained a greater number of samples more than three years old were expected to show a relatively lower average value for the width of the 1944 annual ring. The data in the seventh column in table 2 show that this expectation was confirmed. In the lots containing only three-year-old samples the thickness of the 1944 annual increment was greater than that in the mixed lots, and this difference was particularly conspicuous in the diseased material of the Emperor variety, which contained the largest number of samples over three years old.

Judging by the thickness of xylem and phloem, the Emperor variety produced more vigorous growth than the Palomino, but in tylose development the former showed a greater difference between the diseased and the symptom-free vines than the latter. The high per cent of tyloses in the wood of the symptom-free Palomino probably resulted from the somewhat unfavorable soil and water conditions that prevailed in the Napa Valley vineyard.

A summary of the data in table 2 discloses that, in general, the presence

TABLE 3
CHARACTERISTICS OF VARIOUS TISSUES OF PALOMINO GRAPE VARIETY
AFFECTED WITH PIERCE'S DISEASE

Vine number and kind of sample	Cork of 1945	Connection between bark and wood	Range of thickness of phloem in mm		Per cent	
			Total	Functioning	Of functioning phloem	Of vessels with tyloses in 1945 xylem
Vine 1, mildly diseased						
One-year-old cane	incomplete	weak	0.23-0.64	0.13-0.44	56.5-68.6	1.0
One-year-old cane	incomplete	weak	0.28-0.64	0.15-0.46	53.5-71.9	4.5
One-year-old cane	incomplete	weak	0.13-0.41	0.10-0.28	55.5-68.3	25.2
One-year-old cane	incomplete	weak	0.23-0.48	0.13-0.33	56.5-68.7	28.1
One-year-old cane	incomplete	weak	0.25-0.59	0.10-0.23	38.9-40.0	29.0
Average					52.2-63.5	17.6
Vine 2, moderately diseased						
One-year-old cane	absent	weak	0.16-0.54	0.08-0.36	50.0-66.6	32.0
Two-year-old cane	absent	weak	0.20-0.51	0.10-0.13	25.5-50.0	50.0
Main arm	incomplete	weak	0.41-1.05	0.28-0.36*	26.7-37.8	59.5
Average					34.1-68.1	47.2
Vine 3, moderately diseased						
One-year-old cane		very weak				13.3
Two-year-old cane	absent	very weak	0.41-1.02	0.15-0.38	36.6-37.2	16.9
Three-year-old arm	absent	weak	0.64-0.79	0.15-0.23	28.1-29.1	38.5
Four-year-old arm	absent	weak	0.77-1.02	0.31-0.38	37.2-40.3	35.4
Trunk	incomplete	very weak	0.67-1.31	0.46-0.54*	35.1-80.6	43.3
Average					34.3-46.8	29.5
Vine 4, moderately diseased						
One-year-old cane	absent	weak	0.26-0.51	0.08-0.20	30.8-39.2	9.5
Two-year-old cane	incomplete	weak	0.41-0.87	0.13-0.31	31.7-35.6	43.8
Trunk	incomplete	weak	0.66-1.62	0.56-0.67	41.4-84.8	40.8
Average					34.6-53.2	31.4
Vine 5, moderately diseased						
One-year-old cane	absent	weak	0.38-0.69	0.18-0.46	47.4-66.7	6.8
One-year-old cane	present	weak	0.15-0.56	0.10-0.51	66.7-91.2	7.5
One-year-old cane	incomplete	weak	0.15-0.48	0.10-0.38	66.7-79.2	9.1
One-year-old cane	present	weak	0.20-0.54	0.15-0.51	75.0-94.4	10.8
One-year-old cane	incomplete	weak	0.61-0.82	0.33-0.46	54.1-56.1	16.8
One-year-old cane	present	weak	0.15-0.51	0.10-0.41	66.7-80.4	17.7
One-year-old cane	present	firm	0.18-0.48	0.13-0.38	72.2-79.2	24.4
Two-year-old cane	present	weak	0.23-0.44	0.18-0.36	73.8-81.8	15.2
Two-year-old cane	present	weak	0.15-0.33	0.10-0.23	66.7-69.7	37.4
Two-year-old cane	present	weak	0.16-0.66	0.13-0.61	81.3-92.4	44.9
Trunk	present	firm	0.67-0.79	0.49-0.69	73.1-87.3	39.9
Average					68.0-79.9	20.1

of symptoms was not associated with any marked depression of growth in the arms as evidenced by the small variations (and these were often in favor of the diseased vines) in the thickness of the xylem and phloem tissues. The per cent of functioning phloem in the healthy vines was slightly higher than

TABLE 3—Continued

Vine number and kind of sample	Cork of 1945	Connection between bark and wood	Range of thickness of phloem in mm		Per cent	
			Total	Functioning	Of functioning phloem	Of vessels with tyloses in 1945 xylem
Vine 6, severely diseased						
One-year-old cane	absent	firm	0 15-0 28	0 10-0 18	64.3-66.7	10.5
One-year-old cane	absent	weak	0 20-0 43	0 10-0 28	50.0-65.1	23.4
Two-year-old cane	absent	weak	0 20-0 77	0 10-0 26	33.8-50.0	22.7
Three-year-old cane	absent	weak	0 30-0 80	0 15-0 44	50.0-55.0	19.8
Three-year-old cane	absent	weak	0 51-0 74	0 20-0 28	37.8-39.2	45.1
Trunk	incomplete	very weak	0.66-1.74	0.51-0 72	41.4-77.3	25.8
Average					46 2-58.9	24.6
Vine 7, severely diseased						
One-year-old cane	absent	very weak	0.20-0 51	0 15-0 33	64.7-75.0	32.6
Two-year-old cane	incomplete	weak	0.31-0 92	0 23-0 46	50 0-74.2	67.1
Three-year-old arm	incomplete	weak	0 18-0 92	0 13-0 33	35 7-72.2	59.6
Four-year-old arm	incomplete	weak	0 28-0 52	0 23-0 26	50 0-82.1	59.2
Trunk	uncertain	weak	0.52-0 71	0 26-0 51	50.0-71.9	53.1
Average					50.1-75.1	54 1
Average, vines 1-7					50.8-66.9	29.2
Vine 8, healthy						
One-year-old cane	present	firm	0.28-0 56	0 20-0 46	71.4-82.1	8.9
Two-year-old cane	present	firm	0.25-0 56	0 20-0 46	80.0-82.1	0.2
Trunk	present	firm	0.46-0 64	0 33-0 61	82.6-95.3	0.0
Average					78.0-86.5	3.0
Vine 9, affected with Black Measles						
One-year-old cane	incomplete	firm	0.15-0 48	0 10-0 28	58.3-66.7	3.3
One-year-old cane	present	firm	0.25-0 52	0 20-0 44	80.0-84.5	4.5
One-year-old cane	present	firm	0.08-0 36	0 05-0 28	62.5-77.7	7.3
One-year-old cane	present	firm	0.15-0 39	0 10-0 31	66.7-79.5	7.5
Two-year-old cane	present	firm	0.28-0 62	0 23-0 50	82.2-95.2	0.6
Three-year-old arm	present	firm	0.18-0 56	0 13-0 40	72.2-70.9	0.2
Trunk	uncertain	firm	0 74-0 79	0 56-0 61*	70.2-82.4	0.0
Average					70.4-79 6	3.3

* In these sections the smaller amounts of functioning phloem occurred in the thicker parts of the total phloem. Therefore, in the calculation of the per cent of functioning phloem the higher and the lower values for the latter were considered in reference to the lower and higher values for the total phloem, respectively.

that in the diseased. In contrast, the differences in the per cent of tyloses were striking. These structures were obviously more abundant in the diseased vines than in the checks. Moreover, the symptom-free vines showed an expected increase of per cent of tyloses from the younger to the older wood, whereas in the vines with symptoms this increase occurred toward the younger wood. Thus, table 2 clearly shows that Pierce's disease induces a precocious and excessive tylose development in the affected vines.

In the second series of studies on the effect of Pierce's disease on the xylem and phloem, axes of different ages were compared with one another. The

material for this survey was collected on August 14, 1945, and on September 13, 1945, in vineyards located near St. Helena, California. The varieties Palomino, Carignane, and Petite Sirah were represented in the collections. The diseased vines showed symptoms of different degrees of severity. In addition to those affected with Pierce's disease, two vines showing symptoms of Black Measles (Hewitt *et al.*, 1942b) were sampled. Three healthy vines, one from each variety, served as checks.

Most of the data obtained for the Palomino variety are given in detail in table 3. The first column of this table specifies the kind of axis that was represented by the sample, and the other columns give the characteristics that were determined for the axes of various ages. The following items were considered (table 3): development of new cork; condition of the vascular cambium (column headed by "Connection between bark and wood"); thickness of phloem, with special reference to the relative thickness of the functioning part of the tissue; per cent of vessels with tyloses in the xylem formed in 1945.

Table 3 includes data on seven vines that were affected with Pierce's disease, and showed three degrees of severity of symptoms. One vine was mildly affected; four displayed symptoms intermediate between mild and severe ("moderately diseased" in table 3); and two vines showed severe effects of the disease expressed in dwarfing of the new season's shoots, pronounced mottling and scalding of leaves, underdevelopment and drying of fruit. Vine 8 in table 3 was free of symptoms of any disease, and vine 9 was affected with Black Measles.

When the samples were collected (August), formation of new cork should have taken place, since, under California conditions, this phenomenon usually occurs in July (Esau, 1948a). The item "Cork of 1945" in table 3 shows that the development of new cork was irregular in the diseased vines and that this irregularity occurred in new canes and also in older axes. The 1945 cork was sometimes entirely absent in a given trans-section of the axis, or its development was incomplete, that is, it was present in a part of the section only. Some sections of diseased axes showed normal cork development. The cork was present in all sections from the healthy vine and in most of those from the vine affected with Black Measles.

Divisions had ceased in the vascular cambium in all samples, but whereas in the healthy vines the cambial walls had attained the thickness and firmness characteristic of the dormant state (Esau, 1948a), these walls were still thin and weak in parts or in the entire sections from the vines affected with Pierce's disease. Therefore the connection between the bark and wood was firm in healthy axes—the bark had ceased to slip over the wood—whereas it was weak or very weak in diseased vines. The vines affected with Black Measles showed dormant cambium like the healthy vine. The uneven cork formation and the delay in thickening of cambial walls—as well as the scarcity of storage starch mentioned previously—are indications of incomplete development of the phenomena of dormancy. In other words, the axes of diseased vines do not "ripen" properly toward the end of the season.

The one- to three-year-old canes were represented by entire cross sections, but the samples from the arms and trunks consisted of fractions of the circumference. The thickness of the phloem of a given section was measured in

two regions, the narrowest and the widest. A record of the degree of variation between the thinnest and the thickest portions of the phloem was of interest with regard to the data on the activity of the cork cambium: the presence or absence of cork should have something to do with the thickness of the phloem at the end of the season.

According to table 3, the phloem (total and functioning) fluctuated in thickness in healthy and diseased vines. In some sections from the latter, however, the difference between the thinnest and the thickest parts of the phloem was exceptionally great, and the occurrence of such differences in given sections showed no obvious relation to the presence or absence of cork in the same section. Sometimes the difference in the thickness of phloem depended on the irregular cork development; sometimes it was determined by variations in amount of growth from the cambium in the different parts of a given axis.

The per cent of functioning phloem was generally lower in the vines affected with Pierce's disease than in the healthy vine and in the one showing symptoms of Black Measles. The accumulation of the relatively large amounts of nonfunctioning phloem in the vines with Pierce's disease showed, in this series of samples, a relation to cork activity. Where the latter was absent or was formed incompletely (table 3, vines 1 to 4, 6 and 7) the per cent of functioning phloem was lower, on the average, than in those with normally formed cork (part of samples of vine 5; vines 8 and 9). Thus, the presence of relatively large amounts of nonfunctioning phloem in vines affected with Pierce's disease is, at least partly, another expression of the imperfect development of the dormant state. However, nonoccurrence of reactivation in considerable parts of old phloem and a reduction in cambial growth may also reduce the per cent of functioning phloem.

The tyloses were counted in the same manner as in the first series of studies involving secondary xylem. The primary tracheary elements were included in the counts in the one-year-old canes. In table 3 the excessive tylose development is the most conspicuous distinguishing characteristic of the vines affected with Pierce's disease. The per cent of vessels with tyloses varied considerably in the samples from the same vine, whether the axes represented by the samples from a given vine were of the same age or not. Nevertheless, in the majority of samples the per cent of plugged vessels was higher than the highest per cent in the healthy vine and in the one affected with Black Measles. In the vines with Pierce's disease, the older axes often had more tyloses in the 1945 xylem than the younger ones (see also table 5).

The samples of Petite Sirah and Carignane were surveyed like those of Palomino. In addition to the items listed in table 3, the per cent of tyloses was determined for each annual increment, if more than one of these were present, and the thickness of xylem was measured. Rootstocks from two diseased vines of Palomino and from one of Petite Sirah and some diseased sprouts from Palomino rootstock were examined. (The sprouts showed no tyloses and were omitted from tables 4 and 5.) The studies on Petite Sirah and Carignane gave results similar to those on Palomino (table 3), except that in the average of all samples the former two varieties showed a lower per cent of vessels with tyloses than the latter. The data on Petite Sirah and Carignane are not

given in detail. Instead, the figures obtained with the three varieties are summed up in tables 4 and 5.

Table 4 gives the per cent of vessels with tyloses for each vine sampled. The figures obtained from the axes of different ages and from the various xylem increments were averaged for table 4. The values for the rootstocks were calculated separately from those for the aerial parts. The wood increments of different years showed variable per cent of tylosed vessels. Some-

TABLE 4
COMPARISON OF THREE VARIETIES OF *Vitis vinifera* WITH REGARD TO THE EFFECT
OF PIERCE'S DISEASE UPON THE XYLEM

Vine number	Kind of material	Condition of the vine	Per cent of vessels with tyloses
Palomino			
1	Aerial axes*	Mild symptoms of Pierce's disease	17.6
2	Aerial axes	Moderate symptoms of Pierce's disease	45.2
3	Aerial axes	Moderate symptoms of Pierce's disease	24.5
4	Aerial axes	Moderate symptoms of Pierce's disease	31.4
5	Aerial axes	Moderate symptoms of Pierce's disease	20.9
6	Aerial axes	Severe symptoms of Pierce's disease	25.3
7	Aerial axes	Severe symptoms of Pierce's disease	46.9
10	Rootstock†	Moderate symptoms of Pierce's disease	27.4
7	Rootstock†	Severe symptoms of Pierce's disease	20.8
8	Aerial axes	No symptoms.	5.0
9	Aerial axes	Symptoms of Black Measles.	6.4
Carignane			
1	Aerial axes	Severe symptoms of Pierce's disease	22.6
2	Aerial axes	No symptoms.	3.6
Petite Sirah			
1	Aerial axes	Mild symptoms of Pierce's disease	27.0
2	Aerial axes	Moderate symptoms of Pierce's disease	20.1
3	Aerial axes	Severe symptoms of Pierce's disease	15.0
4	Rootstock†	Moderate symptoms of Pierce's disease	7.9
5	Aerial axes	No symptoms.	5.8
6	Aerial axes	Symptoms of Black Measles.	9.9‡

* Only one-year-old canes were collected from vine 1, axes of various ages from the others.

† Palomino and Petite Sirah were grafted on the same kind of rootstock.

‡ The sample from the trunk of Petite Sirah, vine 6, was infected with a fungus and showed tyloses in 62.4 per cent of vessels. This sample was excluded in the calculation of the average for vine 6.

times the value was higher for the older, sometimes for the younger wood, or all increments were similarly affected. (Table 2, however, shows that when sufficient numbers of samples of a similar kind are examined, the normal increase of the per cent of vessels with tyloses from the younger toward the older xylem increments is mostly reversed in the diseased vines.)

According to table 4, excessive production of tyloses under the influence of Pierce's disease is characteristic of all three varieties considered in the table, and it occurs in the rootstocks, as well as in the aerial parts. No rootstock samples were available from the healthy vines in this series, but, according to some other studies on the roots of the grapevine by the writer (Bead, 1948a), excessive tylose formation is not characteristic of this organ in healthy plants. The samples analyzed in table 4 are not sufficiently numerous for the reader

to judge the relative resistance of the three varieties, as revealed by the severity of vessel occlusion, or to draw conclusions concerning the comparative effects of the disease upon roots and stems. Table 4 reveals no relation between the severity of the external symptoms and the per cent of vessels with tyloses in the xylem.

Table 5 was compiled from the data obtained with the three varieties considered separately in table 4. The values recorded for the axes of each age were averaged regardless of the variety. According to table 5, in axes of all ages the entire phloem was usually thicker in the diseased vines, but the functioning part of the phloem was wider in the healthy vines; and the per cent of functioning phloem was considerably higher in the check plants. The

TABLE 5
COMPARISON OF THE EFFECT OF PIERCE'S DISEASE UPON AXES OF DIFFERENT AGES OF
Vitis vinifera
(Combined averages for all the vines given in table 4)

Kind of axis	Condition of vine	Range of thickness in mm *			Per cent	
		Of total phloem	Of functioning phloem	Of xylem of 1945	Of functioning phloem	Of vessels with tyloses*
One year old.....	Diseased	0.23-0.55	0.13-0.37	0.98-1.67	56.5-67.3	15.2
One year old.....	Healthy	0.19-0.43	0.14-0.35	1.50-2.28	73.7-81.4	3.2
Two to four years old.....	Diseased	0.32-0.70	0.16-0.36	0.46-1.22	50.0-50.1	34.6
Two to four years old.....	Healthy	0.29-0.52	0.24-0.44	0.88-2.09	82.8-84.6	0.1
Trunk.....	Diseased	0.64-1.07	0.44-0.53	1.18-1.51	68.5-49.5	40.1
Trunk.....	Healthy	0.72-0.84	0.61-0.72	0.68-0.95	64.7-85.7	1.5

* Average for xylem of various ages in axes older than one year.

1945 xylem of the younger axes was thicker in the healthy plants, but the trunk xylem of the same year was wider in the diseased plants. The diseased axes, regardless of age, showed a much higher per cent of vessels with tyloses than the healthy material. Among the diseased samples, the one-year-old canes had relatively fewer tyloses than the older axes.

Thus the studies on axes of various ages taken from commercially grown grapevines all agree that Pierce's disease induces excessive and precocious development of tyloses in the xylem, and more or less reduces the per cent of functioning phloem in the bark. Since the thickness of the bark in the diseased vines is frequently greater than in the check plants, the low per cent of functioning phloem in the former seems to depend mainly on irregularities in cork development—that is, excessive amounts of nonfunctioning phloem are left on the vine because of imperfect cork formation.

Development of Symptoms in Seedlings. This study was planned to serve a three-fold purpose: firstly, to determine the nature of the first anatomic changes in the inoculated plants; secondly, to determine the time interval between the inoculation and the appearance of the first external and internal symptoms; thirdly, to compare the spread of the internal and external symptoms through the plant. Three series of seedlings were used. The first two

(tables 6 and 7) were sampled principally before the appearance of external symptoms; the third (table 8) after such symptoms had developed. All seedlings were grown and treated in a greenhouse.

The plants of the first two series were of different sizes. In series 1 (table

TABLE 6
DEVELOPMENT OF SYMPTOMS ON GRAPEVINE SEEDLINGS INOCULATED THROUGH
THE SECOND LEAF ABOVE THE COTYLEDONS

Sample number	Number of days between inoculation and sampling	Number of leaves on plant counted at sampling	Internal symptoms in inoculated leaf*	External symptoms	
				On the day of sampling	Thirteen months after inoculation
1.....	3	5	absent	absent	present
2.....	3	3	absent	absent	present
3.....	†	4	absent	absent	absent
4.....	6	4	absent	absent	absent
5.....	6	4	absent	absent	present
6.....	10	4	absent	absent	absent
7.....	10	5	absent	absent	present
8.....	..	4	absent	absent	absent
9.....	13	5	absent	absent	present
10.....	13	4	absent	absent	present
11.....	17	5	absent	absent	absent
12.....	17	4	absent	absent	absent
13.....	..	4	absent	absent	absent
14.....	24	5	absent	absent	absent
15.....	24	6	present	absent	present
16.....	31	4	present	absent	plant dead
17.....	31	6	absent	absent	present
18.....	..	4	absent	absent	absent
19.....	37	6	absent	absent	present
20.....	37	5	absent	absent	present
21.....	44	6	absent	absent	absent
22.....	44	5	absent	absent	plant dead
23.....	..	5	absent	absent	absent
24.....	51	5	present	absent	present
25.....	51	5	present	absent	present
26.....	58	4	absent	present†	present
27.....	58	5	absent	present†	present
28.....	..	5	absent	absent	absent
29.....	66	6	present	present	absent
30.....	66	5	present	present	uncertain

* If internal symptoms were present, they occurred in the inoculated leaf, but were absent in the other parts sampled.

† The plants for which the item "Number of days between inoculation and sampling" is not recorded are untreated control plants. In each instance, the control plant was sampled together with the two plants listed just above it in the table.

‡ The external symptoms on these two plants were observed 3 days before sampling—that is, 55 days after inoculation.

6) the seedlings had three to four leaves above the cotyledons at the time of inoculation (September 8, 1944), and the infective insects were caged on the second leaf above the cotyledons. In series 2 (table 7) the plants had nine to thirteen leaves above the cotyledons at inoculation (September 13, 1944), and the insects were placed on the third or fourth leaves from the top. Ten insects (adult *Draeculacephala minerva* Ball) were placed on each plant for 24 hours. Alfalfa plants affected with Pierce's disease virus served as a source of the inoculum.

In series 1 and 2 the first collections were made a few days after the infective leafhoppers were placed on the plants. The samplings were repeated at first every few days, and later once a week (see column "Number of days between inoculation and sampling" in tables 6 and 7). On each day of sampling, two

TABLE 7
DEVELOPMENT OF SYMPTOMS ON LARGE GRAPEVINE SEEDLINGS INOCULATED
THROUGH THE THIRD OR FOURTH LEAVES FROM THE TOP

Sample number	Number of days between inoculation and sampling	Number of leaves on plant counted at sampling	Internal symptoms in inoculated leaf*	External symptoms	
				On the day of sampling	Thirteen months after inoculation
1.	2	11	absent	absent	absent
2.	2	13	absent	absent	absent
3.	†	12	absent	absent	absent
4.	5	8	absent	absent	present
5.	5	13	absent	absent	absent
6.	8	14	absent	absent	absent
7.	8	14	absent	absent	absent
8.	11	absent	absent	absent
9.	12	13	absent	absent	absent
10.	12	13	present	absent	absent
11.	15	15	absent	absent	present
12.	15	11	absent	absent	absent
13.	12	absent	absent	absent
14.	19	14	absent	uncertain†	present
15.	19	11	absent	absent	absent
16.	26	15	absent	absent	absent
17.	26	16	absent	absent	present
18.	12	absent	absent	absent
19.	33	14	present	absent	absent
20.	33	11	present	absent	absent
21.	40	12	present	absent	absent
22.	40	11	absent	absent	absent
23.	10	absent	absent	absent
24.	47	11	present	absent	present
25.	47	12	present	absent	present
26.	54	12	present	absent	present
27.	54	13	present	absent	present
28.	11	absent	absent	absent
29.	62	12	present	present	present
30.	62	11	absent	present	present
31.	74	13	present	present‡	present

* If internal symptoms were present, they occurred in the inoculated leaf, but were absent in the other parts sampled.

† The plants for which the item "Number of days between inoculation and sampling" is not recorded are untreated control plants. In each instance the control plant was sampled together with the two plants listed just above it in the table.

‡ In these two plants the main shoot apices were dead and, therefore, axillary buds were sampled for the study of apical meristems.

diseased plants were collected in each of the two series, and every other sampling day a healthy plant was included in the collection. (The samples from the healthy plants are numbered 3, 8, 13, 18, 23, and 28 in tables 6 and 7.) The samples were taken from four different parts of each plant: 1) petiole of the inoculated leaf; 2) internode below the inoculated leaf; 3) internode above the inoculated leaf; and 4) shoot apex with part of the uppermost elongated internode. The portion of the plant remaining after sampling was left undisturbed, and was examined for the development of external symp-

toms about 13 months after the inoculation (October 8, 1945). The results of this examination appear in the last columns of tables 6 and 7.

In series 1 and 2, internal symptoms occurred in few plants (column "Internal symptoms in inoculated leaf"), and among the parts sampled only the petioles of the inoculated leaves showed these symptoms. The pathologic change was localized in the xylem, and consisted mainly of the presence of gum in vessels of various ages, although tyloses also occurred. When present, the gum commonly extended for considerable distances longitudinally in a given vessel, and therefore many successive serial sections from one piece of petiole showed the symptoms. Occasionally, tyloses and small amounts of gum occurred in a few sections on a slide (in series 2, scattered gum formation was found in two of the healthy plants), but such localized plugging of xylem elements was not interpreted as evidence of the disease. Tyloses that occurred in the oldest xylem of the internodes of some healthy and diseased plants were judged to be normal formations associated with the cessation of function of the first-formed xylem.

No other tissues beside the xylem showed any abnormalities in the infected plants, and the apical meristems of the latter did not differ anatomically from those of the untreated plants, except that in series 2 the apices of two plants had died before the sampling was made (samples 14 and 31 in table 7). In many plants, the older of the two or both internodes showed cork formation that was characterized by no abnormalities. The external symptoms were discolorations and necroses on parts of leaf blades.

According to the data in tables 6 and 7, the internal symptoms developed before the external ones. Some plants having gum in the xylem at sampling time failed to develop any external symptoms 13 months after the inoculation. Sometimes the presence of external symptoms was associated with no anatomic changes in the parts sampled. Among the older seedlings the number of plants that eventually showed internal symptoms was higher than among the younger seedlings, but a higher number of the younger seedlings developed external symptoms. The symptom development was irregular in each lot. No explanation of this behavior may be offered at present. Although the seedlings were not uniform genotypically and were expected to show somewhat variable behavior in their response to infection, they all belonged to the species *vimifera*, which is generally very susceptible to the disease.

In the younger seedlings, the first internal symptom was observed 24 days (sample 15 in table 6), and the first external symptom 55 days after the inoculation (samples 26 and 27 in table 6). In the older seedlings, the first anatomic change was noted 12 days after inoculation (sample 10 in table 7). If the death of the apical meristem in plant 14 in table 7 resulted from the disease, then the first external evidence of the latter appeared in the series of older seedlings 19 days after the inoculation (plant 14, table 7). The external symptoms on the inoculated leaf developed in this series 62 days after the inoculation (plant 29 in table 7).

Judging from the last column in tables 6 and 7, Pierce's disease virus certainly spreads downward from the inoculated leaf: although this leaf and the internode below it were removed at sampling, in many instances the remaining stub of the plant developed external symptoms on the new growth. In two

of the small seedlings (samples 1 and 2 in table 6) the virus was present in the base of the plant 3 days after inoculation. However, in general the virus appeared to move slowly out of the inoculated leaf and was therefore frequently removed with the sample, particularly in the plants sampled in the earlier part of the experiment. (See tables 6 and 7, last column.)

The seedlings of the third series, which were used for a comparison of the development of the external and internal symptoms (table 8), had three to four leaves when they were inoculated. The vectors (ten adult *Neokolla cir-cellata* Baker on each plant) were placed for 24 hours usually on the second leaf above the cotyledons, sometimes on the first. The plants were inoculated on January 17 and 18, 1945. The date of appearance of the first external symptoms was recorded for each plant, and the collections for microscopic study were made at different times after these dates. Since series 1 and 2 included healthy material in developmental stages comparable with those of the plants in series 3, no noninoculated seedlings were included in the latter. When sampled, the plants were dissected as for series 1 and 2; in addition, a piece of the midvein with the adjacent mesophyll of the inoculated leaf was collected from each plant. Furthermore, certain other parts were sampled in some plants.

As in series 1 and 2, the plants in series 3 usually showed the first external symptoms on the inoculated leaf. Sample 16 of series 3 was exceptional in that no external symptoms developed on this leaf. The plants of series 3, which were inoculated in the month of January, developed the first symptoms sooner than the plants in series 1 and 2, which were treated in the early fall. Perhaps the better light conditions in the greenhouse in January (because of absence of whitewash on the windowpanes) as compared with those in the early fall (when the windows were coated with whitewash) speeded up the development of symptoms in series 3.

The seventh column in table 8 indicates the manner of spread of the external symptoms beyond the inoculated leaf. The external symptoms developed not only on the leaves located above the inoculated leaf, but also in the leaf below it. Similarly, the internal symptoms spread downward and upward from the inoculated leaf (eighth column in table 8). These observations, together with the data presented in tables 6 and 7 indicate that the infective principle of Pierce's disease moves downward as well as upward in a plant.

The data on the shoot apices were not included in tables 6 to 8. This was not done because, as was mentioned previously, no abnormalities were detected in the apical meristem or the subjacent region in diseased plants, except, of course, in the instances when the shoot tips became necrotic.

The observation that the earliest symptom of Pierce's disease in grape seedlings occurred in the xylem, that it appeared first of all in the inoculated leaf, and that it developed before the external evidences of the disease, suggests that gummosis of the xylem is a primary symptom. Judging by the location of the first internal symptoms (in the inoculated leaf; and then in the well-developed internodes below it and above) Pierce's disease induces degeneration changes in plant parts that are in advanced stages of development.

Additional evidence that xylem symptoms are primary in nature was

TABLE 8
COMPARISON OF THE DEVELOPMENT OF EXTERNAL AND INTERNAL SYMPTOMS
IN GRAPEVINE SEEDLINGS

Sample number	No. of days between the different events			Number of leaves above the inoculated, counted at sampling	Description of external symptoms on the date of sampling		Internal symptoms in certain parts other than the inoculated leaf*
	Inoculation and appearance of external symptoms	Inoculation and appearance of external symptoms	Appearance of external symptoms and sampling		On the inoculated leaf	On leaves other than the inoculated	
1	71	36	35	2	Red discoloration along margin; necrosis at apex	None	None
2	71	42	29	2	Red discoloration along margin and in spots on blade; some necrosis	None	Possibly in internodes 1† and 2
3	70	50	20	2	Necrosis along margin	None	None
4	71	51	20	3	Necrosis along margin	None	None
5	70	52	18	2	Red discoloration and necrosis along margin	None	Possibly in internode 1
6	71	53	18	2	Red discoloration and necrosis along margin	None	Possibly in internode 1
7	70	55	15	2	Red discoloration and necrosis along margin	None	Possibly in internode 1
8	70	59	11	3	Necrosis at apex	None	None
9	71	64	7	2	Necrosis at apex	None	Possibly in internode 1
10	71	64	7	3	Yellowish discoloration throughout	Necrotic spot on leaf —†; necrosis along margin of leaf 2	In internodes 1 and 2
11	71	66	5	4	Necrosis at apex	None	None

12	70	65	5	2	Nerosis at apex	None	Possibly in inter-nodes 1 and 2
13	83	59	24	5	Nerotic margin	Nerotic margin on leaf —1; necrotic margins and spots on leaves 3 to 6	None
14	84	64	20	5	Nerotic margin	Nerotic margin on leaf 3; necrotic spot on leaf 6	None
15	84	66	18	3	Nerotic margin	Yellowish discoloration of leaf —1; necrotic spot on leaf 3	None
16	83	75	8	6	None	Yellowish discoloration of and necrotic spots on leaves 5 to 7	None
17	83	63	20	3	Nerosis almost throughout	Nerotic margin on leaf 2; one necrotic spot on leaf 3	None
18	83	65	18	5	Yellowish discoloration throughout	Yellowish discoloration of and necrotic margin on leaf 2; necrosis beginning on leaf 3	Possibly in inter-nodes 1 to 4 and petioles 3 and 5
19	92	63	29	6	Nerosis of half of leaf blade	Yellowish discoloration of and necrotic spots on leaves 2 and 3; crinkly, up-turned margin on leaf 3; necrotic spots on leaves 5 and 6	In internodes 1 and 2
20	92	63	26	4	Nerosis of large areas	Nerosis at apex of leaf —1; necrotic spot on leaf 3	In internode 1
21	93	53	40	3	Red discoloration along margin	Nerotic spots on leaves 2 to 4	None
22	92	82	10	4	Nerosis at apex	None	None

* The inoculated leaf showed internal symptoms in all 22 plants; they had more or less abundant gum and some tyloses in the sections of midveins and petioles.
† The inoculated leaf and the internode below it were given the number 1. The leaves and internodes above the inoculated leaf were numbered 2, 3, etc., beginning with the lowest. The leaf below the inoculated one was numbered —1.

obtained from studies on xylem degeneration in relation to the feeding punctures of the vectors in some seedlings sampled soon after the viruliferous and virus-free insects had fed upon them. Tyloses and gumlike deposits sometimes occurred in the xylem near the feeding punctures made by noninfective leafhoppers in healthy plants. Moreover, some of the punctured vessels were crushed by wound-reaction growth. If, however, infective leafhoppers fed upon healthy plants, gum and tyloses developed not only near the puncture but also at some distance from it. Plate 5, *A*, for example, shows a 14-day-old feeding puncture (torn in the cambial region by wound-healing growth) made by *Draeculacephala minerva* in a certain bundle, and plate 5, *B*, illustrates the condition of the same bundle 750 microns from the feeding puncture. The gum and tyloses in the xylem in plate 5, *B*, seem to have been formed not merely in response to the injury by the insect, but as a first symptom of Pierce's disease.

Effect of the Disease on New Growth on Old Vines. Six shoots with pronounced leaf symptoms, obtained from four vines of the Emperor variety and two vines of a Muscat variety, were compared with healthy shoots of similar stage of development. The collections were made in the spring and early summer. The apex and pieces from four to seven internodes and leaves were available from each shoot, and in the preparation for fixation the position with respect to the apex was recorded for each severed internode and leaf. In each shoot, the search for internal symptoms was made in the apical portion and in the successively older internodes, leaf blades, and petioles.

Pathologic changes were observed in the xylem and the mesophyll; none was observed in the phloem. The xylem showed mainly an occlusion of some vessels with gum. Tyloses occurred in obliterating elements, probably as part of the normal phenomenon of obliteration, since such tyloses were common in healthy material also. The symptoms in the mesophyll were those described in a preceding part of this paper.

In general, gum development in the xylem was scattered in different parts of the shoot and was not at all severe. It was most common in the protoxylem of the apical part of the shoot, in the young leaves and internodes, and in the minor veins of the older leaves. The internodes, the petioles, and the major veins of the blades from the older parts of the shoot rarely showed gum. The protoxylem of the older shoot parts appeared to have been gummed before the obliteration, but the subsequently formed xylem was normal in appearance. Perhaps the occlusion of the protoxylem is the cause of occasional necrosis of shoot apices in affected plants.

Chloroplast degeneration in the mesophyll was evident only in the older leaves, and here it occurred in spots. Both the green and the yellow-leaf areas showed abnormalities, but the green mesophyll was less severely affected.

PATHOLOGICAL ANATOMY OF ALFALFA AFFECTED WITH THE DWARF DISEASE

Structure of Roots of Healthy Alfalfa. Taproots in the secondary stage of growth, tips of lateral roots, and bases of stems were examined for the effects of Pierce's disease virus upon alfalfa. The tips of lateral roots of healthy plants show the usual primary structure characteristic of dicotyledonous

roots. The cortex is wide, is composed of parenchyma, and covered by an epidermis. The narrow central cylinder has diarch, triarch, or tetrarch xylem, two to four phloem strands, and a parenchymatous pericycle which bounds the periphery of the stele. (Compare Simonds, 1935, and Hayward, 1938, p. 322.) The endodermis is characterized by the presence of Casparian strips. The order of differentiation of the xylem, phloem, and endodermis with respect to the apical meristem is considered in connection with the description of root tips of diseased plants.

The taproot in secondary stage of growth is well described and illustrated by Simonds (1935) and Jones (1928a). Like other dicotyledonous roots, this organ of the alfalfa plant loses its cortex in the early stages of secondary growth. The pericycle gives rise to a periderm, with orderly arranged cork cells forming the surface of the root (fig. 1, A). Beneath the periderm is the phloem, mostly of secondary origin. Functioning sieve tubes occur only near the cambium; elsewhere these elements are obliterated, and the phloem region contains only storage parenchyma and phloem fibers.

The xylem, as seen in figure 1, A (inside of the cambium at *a*) is mostly secondary in origin. Some primary xylem occurs in the center of the root, but in old taproots this region is modified as compared with its original state: the xylem parenchyma is much dilated, and the primary-xylem strands are broken up and partly crushed. The secondary xylem contains vessels of different diameters, parenchyma, and numerous fibers, which are lignified in old roots. The vessel elements are mostly scalariform and reticulate. Both the xylem and the phloem show stranded condition because of the presence of wide parenchymatous vascular rays (fig. 1). According to Jones (1928a), the secondary-vascular tissues may be produced in very orderly manner, each season's growth beginning with a fibrous layer and ending with tissue containing the conducting elements. In such roots, the annual increments are readily distinguished when the organs become several years old. The material used in the present study showed no particularly orderly alternation of fibers and other tissue elements.

The alfalfa stem retains its cortex and epidermis in the secondary state. The vascular tissues, at first in discrete strands, later become more or less continuous through the activity of the vascular cambium. A parenchymatous pith is enclosed within the vascular cylinder. Prominent caps of phloem fibers delimit the phloem on the outside. In old stems, active sieve tubes occur only near the cambium. Vessels of different diameters occur in the xylem.

Internal Symptoms of Alfalfa Dwarf Disease. As Weimer (1931, 1936) has stated, the characteristic symptom of alfalfa dwarf is the formation of gum in the xylem (fig. 1, B, at *c*; plate 15). The gum occurs in stems (plate 15, A), at least in their basal portions, and in roots (fig. 1, B; plate 15, B and C). Longitudinal views show the gum filling the vessels for considerable distances. This substance may occur in the parenchyma and fibers adjacent to the affected vessels. In advanced stages of gummosis, partial dissolution of walls leads to the formation of gum pockets. The phloem shows no abnormalities.

The appearance, staining reactions, and the distribution of the gum in the xylem of alfalfa resemble those in the affected grapevine. Plate 15, A, shows several vessels with gum in an alfalfa stem. Some of the gum in this photo-

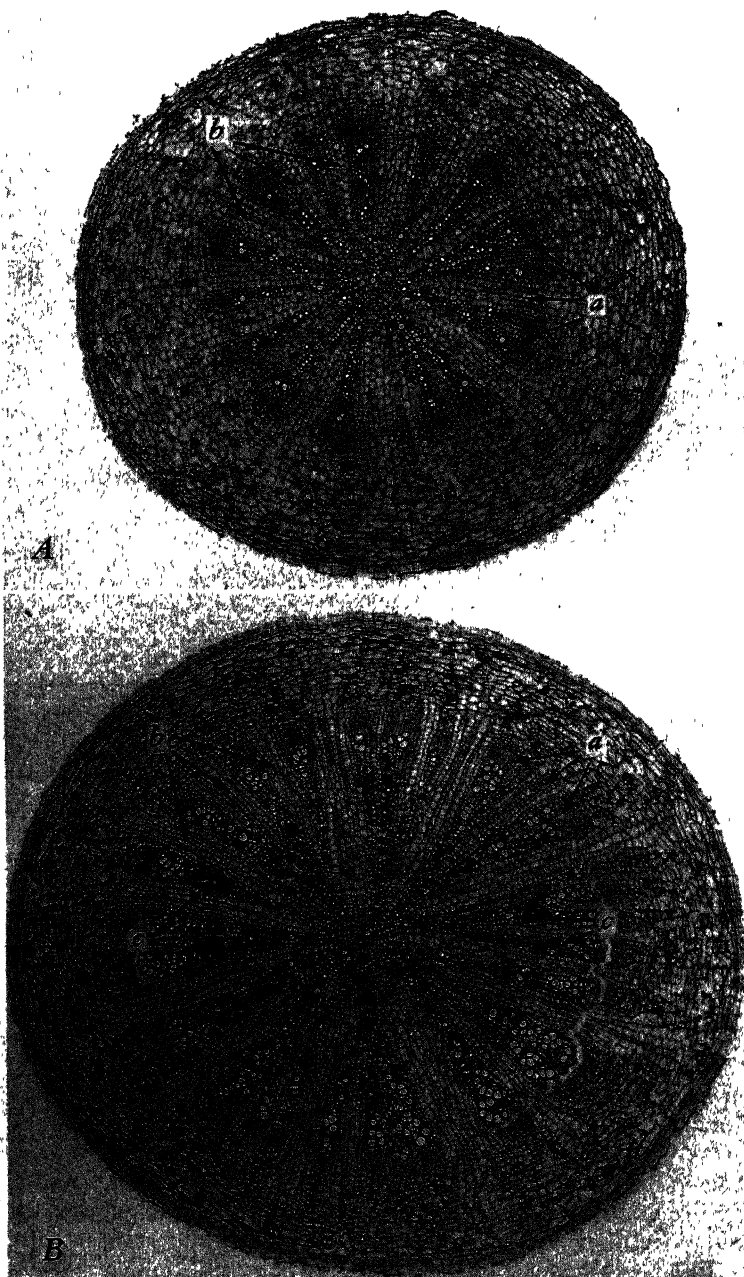


Fig. 1.—Transverse sections of alfalfa roots from a healthy plant (A), and from a plant affected with alfalfa dwarf disease (B). The regions of the root from outside in are: periderm, secondary phloem, cambium (a), secondary xylem, primary xylem. At b are some xylem rays. In B many vessels are occluded with gum (c). (Both $\times 30$.)

graph is stained green and yellow, some red. The latter type of gum is closely appressed to the secondary wall, and shows the same deep staining as the swollen pit-closing membranes which appear like thick red dashes between certain cells. The secondary walls are difficult to discern in the gummed vessels, but wherever visible they show a rather pale coloration. The stains ordinarily used for bringing out lignified walls do not clearly differentiate between the red-staining gum and the similarly tinted walls. Acid fuchsin, however, makes such differentiation possible because it is readily absorbed by the gum and the disorganizing walls, but not so by the apparently unaffected lignified walls. Thus, the views *B* and *C* in plate 15 clearly differentiate between the gum and the swollen pit-closing membranes on the one hand and the secondary wall on the other. Plate 15, *C* also shows, in the lower part of the figure, the disintegration of some parenchyma walls. In the lower right-hand corner of the figure, a swollen wall has pushed part way into the lumen of a parenchyma cell.

To ascertain the causal relation between infection with Pierce's disease virus and gummosis in the xylem, the development of the internal symptom was followed in a series of plants that were sampled at certain known intervals after exposure to feeding by infective vectors. One-month-old plants of the California Common alfalfa variety were transplanted into pots and kept in a greenhouse. When the plants were 2 months old, they were inoculated with Pierce's disease virus by the use of adult *Draeculacephala minerva*. Ten insects were caged upon each plant and were left to feed on it for 2 days. The check plants were left noninoculated, but otherwise were treated just like the inoculated plants. None of the plants developed any external symptoms. Alfalfa affected by the dwarf disease shows no leaf mottling, and the depression of growth characteristic of the disease requires a long time to become evident.

The results of the microscopic studies on the alfalfa plants are summarized in table 9. At successively later periods after the inoculation, series of plants were dug, and root and stem pieces were killed in a mixture of formalin, acetic acid, and alcohol. Each series included two to five plants, mostly four (table 9, first column). The second column in table 9 specifies the contents of the samples from each plant, and the third column gives the observations on occurrence of gum in the xylem of the various samples.

According to table 9, some gummosis was detected 100 hours after the completion of the inoculation procedure. On the succeeding sampling dates, gummosis became more and more pronounced and more widespread. Eight weeks after the inoculation, most of the plants of a series showed severe effects of the disease. One of the control plants (series 10, table 9) had some vessels with gum. The cause of this abnormality was not ascertained, but an accidental infection with Pierce's disease virus was not likely to have occurred because all necessary precautions were taken to prevent the escape of insects.

Thus the data in table 9 clearly show that gummosis in the xylem is associated with alfalfa dwarf, and suggest that it is a primary symptom of the disease.

The root tips were examined for condition of the apical meristem and the course of differentiation of the first vascular elements. There were 118 root

TABLE 9
DEVELOPMENT OF SYMPTOMS IN ALFALFA PLANTS INOCULATED
WITH PIERCE'S DISEASE VIRUS

Series and plant number	Parts of plant represented in sample	Vessels with gum
Series 1—20 hours after insects were removed from plant:		
1.....	Taproot and one stem	None
2.....	Taproot and four stems	None
3.....	Taproot and three stems	None
4.....	Taproot and four stems	None
Series 2—48 hours after insects were removed from plant:		
1.....	Taproot and four stems	None
2.....	Taproot and four stems	None
3.....	Taproot and three stems	None
Series 3—100 hours after insects were removed from plant:		
1.....	Taproot* and four stems	None
2.....	Taproot and four stems	None
3.....	Taproot* and four stems	Few in two stems
4.....	Lateral root and four stems	None
Series 4—1 week after insects were removed from plant:		
1.....	Taproot and four stems	Few in two stems
2.....	Taproot and four stems	Few in one stem
3.....	Taproot and four stems	Three in root, few in three stems
4.....	Taproot and four stems	None
Series 5—2 weeks after insects were removed from plant:		
1.....	Taproot and four stems	None
2.....	Taproot and four stems	Few in one stem; many in two stems
3.....	Taproot and four stems	None
4.....	Taproot and four stems	None
Series 6—4 weeks after insects were removed from plant:		
1.....	Taproot* and three stems	None
2.....	Taproot and three stems	Few in root; one in one stem
3.....	Taproot and two stems	Few in root
4.....	Lateral root and two stems	None
Series 7—6 weeks after insects were removed from plant:		
1.....	Taproot and four stems	Many in all parts
2.....	Taproot and four stems	Few in three stems
3.....	Taproot and four stems	Few in three stems
4.....	Taproot and four stems	Few in root and three stems; many in one stem
Series 8—8 weeks after insects were removed from plant:		
1.....	Taproot and four stems	Many in root; few in four stems
2.....	Taproot and four stems	Many in root and two stems; few in one stem; very many in one stem
3.....	Taproot and three stems	Many in root and one stem; few in one stem
4.....	Taproot and four stems	Many to very many in all parts
5.....	Taproot and three stems	Few in all parts
Series 9—untreated; plants of same age as in series 6:		
1.....	Taproot and one stem	None
2.....	Taproot and three stems	None
Series 10—untreated; plants of same age as in series 8:		
1.....	Taproot and four stems	One to two in root and three stems
2.....	Taproot and three stems	None
3.....	Taproot and two stems	None
4.....	Taproot and four stems	None

* Each of these taproot sections represented only one half of the entire root. All the other taproots and all stems were represented by entire trans-sections.

tips from diseased plants and 29 from healthy. In both groups, some root tips showed entirely normal structural details, such as dense cytoplasm in the apical meristem; presence of the first sieve tubes at a much shorter distance from the apical meristem than the first xylem; presence of endodermis with Casparian strips at the same level as the xylem. Other healthy and diseased root tips displayed symptoms of inactive growth. The apical-meristem cells were more highly vacuolated than in the first group of root tips; sometimes

TABLE 10
COMPARISON OF HEALTHY AND DISEASED ALFALFA ROOTS WITH
REFERENCE TO STARCH STORAGE AND GUM FORMATION

Plant number	Rating of the two phenomena in healthy plants*		Rating of the two phenomena in diseased plants*	
	Starch storage	Gum formation	Starch storage	Gum formation
1	5	0	1	1
2	5	0	0	1
3	5	0	1	2
4	5	0	2	2
5	4	1	0	4
6	4	0	0	4
7	3	0	0	5
8	2	3	2	5
9	4	0	2	3
10	5	0	1	3
11	5	0	3	3
12	5	0	2	2
13	4	1	1	3
14	5	1	0	4
15	3	0	2	4
16	5	0	3	4
17	4	0	1	4
18	5	4	1	4
19	5	0	3	4
20	3	0	2	1
Average	4.3	0.5	1.4	3.2

* Qualitative rating: 0, indicating absence; 5, the largest amount of starch or gum.

they appeared hardly less dense than the differentiating cortical cells. The xylem and phloem elements were mature very close to the apex, and both at nearly the same distance from the apical meristem. The xylem elements were of the type usually differentiating in organs that had just ceased to elongate, namely, scalariform and reticulate, whereas in the active root tips the first xylem elements were with spiral thickenings. The endodermis with Casparian strips occurred as close to the apex as the xylem.

The features listed above indicated that some root tips were retarded in their growth, probably because of the topping and transplanting operations. Plants sampled at the latest dates had some root tips in which renewed growth had occurred after a period of retardation. The new growth was clearly set off from the old, and had the normal characteristics of actively growing root tips. In diseased plants, 72.0 per cent of the root tips were more or less retarded, in the healthy, 55.2 per cent. Since the number of healthy root tips

was relatively small, this difference in per cent may not be significant. Four of the 118 root tips from the diseased plants had gum in the xylem. The same abnormality occurred in two of the 29 healthy root tips. There was no evidence of degenerative changes in the phloem in any root tips.

Thus, the root apices of the diseased alfalfa plants used in the present study revealed no anatomic effects that could serve as diagnostic features of alfalfa dwarf. Similar observations were made on the shoot apices of grape seedlings affected with Pierce's disease. Perhaps some special cytological techniques, for example, a staining according to McWhorter (1941), would reveal differences between healthy and diseased plants other than the gummosis in the xylem, but such techniques were not used in the present study.

Since Pierce's disease virus was known to cause a reduction in the amount of starch stored in the canes of the grapevine, a similar effect was expected in the taproots of alfalfa. To check the relation between presence of disease and starch storage in alfalfa, taproots from twenty healthy and twenty diseased field-grown plants were compared for occurrence of starch and gum.

The plants were dug on June 24, 1947, and the roots were preserved in 50 per cent alcohol. The sections were made with a freezing microtome, and stained with iodine for the study of starch deposition and with phloroglucinol and hydrochloric acid for the identification of gummosis. Table 10 summarizes the observations. The amount of starch and gum was estimated qualitatively by using a rating of from 0 to 5 to characterize the roots with the different amounts of these substances. The absence of either gum or starch was indicated by a 0, the highest amounts with a 5. Table 10 shows that most of the roots of the healthy plants contained much starch, but that only a few of these had gum in the xylem. In contrast, the diseased plants stored none or small amounts of starch in the roots, and most of them showed pronounced to severe gummosis. According to the literature, gum is derived to a large extent from hydrolyzed starch. Perhaps some of the starch in the diseased alfalfa roots has been used up in the formation of gum, at least in the immediate vicinity of the gummed vessels. However, it is also possible that the disease adversely affects the elaboration and storage of carbohydrates through disturbances in the water-conducting system. Table 10, at least, reveals no close correlation between the degree of starch depletion and the severity of gummosis.

EFFECT OF PHONY DISEASE ON PEACH ROOTS

As mentioned in the review of literature, the standard test for the identification of phony disease in peach roots—treatment of fresh sections with acidulated methyl alcohol—indicates that some changes occur in the root xylem of affected trees. Following the techniques employed previously in the study of the grapevine anatomy (Esau, 1948a), sections of roots from healthy and diseased trees were prepared for microscopic study. Both the xylem and the phloem were examined.

The phloem of the peach has been described in detail by Schneider (1945). The roots from healthy and diseased trees used in the present investigation did not differ in the structure of this tissue. In both kinds of roots the sieve tubes expanded normally in the process of maturation, developed thick (macré)

walls, and became crushed after their function ceased. The crushed sieve tubes showed no red coloration when treated with phloroglucinol and hydrochloric acid.

According to Burgerstein (1899), the xylem of most Pruneae is diffuse porous, but is ring porous in *Prunus persica*. This feature is probably variable; at least, the peach roots used in the present study had a diffuse porous wood (plate 16, *A*) or a wood that was indistinctly ring porous (plate 16, *B*). The vessels occur singly, or in radial rows, or in groups (plates 12, *B*, and 16, *A*). They have bordered pits and more or less pronounced spiral thickenings over the secondary wall. Strasburger (1891) and Burgerstein (1899) classified the narrow sclerified elements of the xylem (the small thick-walled cells among the vessel in plate 12, *B*) in the representatives of Pruneae as fiber-tracheids and tracheids, respectively, and both authors found no fibers in these plants. The tracheids have bordered pits. The living cells of the peach xylem are largely confined to the rays (plates 12 and 16). The xylem parenchyma is very sparse (plate 13, cells marked with *a b*).

The sections of roots from the phony peach consistently showed variable numbers of gummed areas in the xylem. The gum, granular or homogeneous, partly or entirely filled the vessel and was also present in the tracheids, xylem parenchyma, and ray cells (plates 12, *A*, and 13, *A*). If gum occurred in the ray cells, the latter were partly or entirely depleted of storage starch. (Compare *A* and *B* in plate 12.) In the gummed areas the pit-closing membranes of the water-conducting cells were swollen and of the same color as the gum. (Compare *A* with *B* and *C* in plate 13.) Tyloses occurred in few vessels in the affected areas.

Occasionally gum pockets were present in the xylem, and the orderly localization of these formations in the oldest part of an annual ring (plate 16, *B*, *a*) indicated that degeneration occurred at the beginning of the growth season. (One stem section from a healthy tree showed similar gum pockets in the oldest part of the secondary xylem.) Usually the rays are not destroyed in the formation of the pockets, but often they contain gum in such areas (plate 16, *B*). A similar pattern of gummous degeneration was observed by Webber and Fawcett (1935) in psorosis-affected citrus and by Wigand (1863) in *Prunus avium* suffering from gummosis. Because the gum-containing cavities in citrus are much elongated vertically and anastomose with each other, Webber and Fawcett call them "gum ducts," and suggest that undifferentiated xylem cells are involved in their formation. Butler (1911) characterizes the cells that break down with formation of gum in *Prunus* and *Citrus* affected with gummosis as "a susceptible tissue, in reality embryonic wood cells." According to Wigand (1863), gummous degeneration affects the abnormal strands of parenchyma that are produced by the cambium instead of the normal elements of the longitudinal system. The origin of the gum pockets in phony peach was not investigated in this study. The references just mentioned show that the formation of the orderly arranged gum pockets in the earliest part of an annual ring may be induced by disturbances of various origin, and that it indicates an abnormal behavior of the cambium at the inception of growth in the spring. The size and course of the gum pockets in longitudinal sections were not studied in the peach material.

DISCUSSION

Pierce's disease of the grapevine, alfalfa dwarf, and phony disease of peach may be grouped with the psorosis of citrus as virus diseases causing degenerative changes in the xylem. Moreover, in all four diseases these changes involve formation of gum and its deposition in vessels and in other xylem cells. As was pointed out in the review of literature, gummosis in xylem is not a very specific symptom because it may be caused by various agencies other than viruses.

The details of the gummosis are very similar in the diseases induced by the viruses of Pierce's disease and phony peach. The gum occurs in all types of xylem cells and, if present in the vessels, it fills them longitudinally for considerable distances. (In psorosis-diseased citrus the gum is limited, in the vessels, to the perforation-plate areas.) In the affected xylem of the grape, alfalfa, and peach, the pit-closing membranes often become swollen and discolored. A partial breakdown of cell walls was observed in alfalfa roots, and their complete disappearance, in the formation of gum pockets, in the peach.

The relation of starch to gum has not been clarified in this study. Only the peach material suggested that starch could be the source of gum, since ray cells filled with gum had little starch, whereas unaffected ray cells of the same sections contained abundant starch (plate 12). In all three kinds of plants, the gum showed various gradations of color when treated with phloroglucinol and hydrochloric acid: yellow, orange, and red. With the same reagents, the swollen pit-closing membranes stained a deep red of similar quality as in the red-colored gum.

Whereas in the young axes and in the leaves of the grapevine the deposition of gum in the vessels was the outstanding abnormality, tylose formation was the more striking phenomenon in the older axes. Some few tyloses were observed in the peach, but none in the alfalfa. Since the grapevine readily produces tyloses under the influence of various injuries and in the normal course of ageing of the wood, some specific characteristic of this plant might be responsible for the abundant development of tyloses in response to an infection with Pierce's disease. Ráthay (1896) observed that in cut grapecanes, tyloses developed subsequently to the occlusion of the injured vessels with gum. Perhaps the tylose development in vines affected with Pierce's disease is also a secondary reaction following the formation of gum; at least, the observations on the recently inoculated grape seedlings suggest this sequence of symptom development.

Although Pierce's disease of the grape and dwarf disease of alfalfa are caused by the same virus, the leaf symptoms differ in the two plants: the leaves of the affected grape are mottled, those of the alfalfa are not. No satisfactory explanation of this difference is at present available. The mottling of grape leaves develops in the second or later seasons of infection with Pierce's disease, and may be a purely secondary symptom. Some recent work on virus effects has shown that mottling of leaves may be caused by the virus not directly but through products of a deranged metabolism (Mckinney and Hills, 1941; Wynd, 1943). If the mottling in the grape also is induced by substances other than the virus itself, the absence of such mottling in the alfalfa might be

determined by some distinguishing features of the metabolism in this plant.

In the seedlings of the grape and the cuttings of alfalfa the first symptoms were observed in the more or less developed parts of plants, rather than in the differentiating organs. Although the older literature stresses that viruses induce symptoms mainly in plant parts that are immature when the virus invades them, sufficient evidence has now been accumulated to indicate that such symptoms appear on fully developed organs, also, if the virus succeeds in entering the latter. (See review by Esau, 1948*b*.)

Despite the general similarity of the anatomic symptoms in the diseases caused by the viruses of Pierce's disease, alfalfa dwarf, phony peach, and psorosis of citrus, the tissue relations of these viruses may not be alike. The manner in which Pierce's disease virus is transmitted to its hosts—it must be introduced into the xylem before it can multiply and cause infection (Houston *et al.*, 1947)—clearly points toward a close relation between this virus and the xylem tissue; in contrast, the psorosis virus is readily transmitted through bark grafts (Wallace, 1945). Moreover, the xylem symptoms induced by Pierce's disease virus appear to be primary in origin, whereas characteristic extra xylary symptoms develop more or less in advance of the xylem abnormalities in psorosis-affected citrus (Webber and Fawcett, 1935). Thus, the psorosis virus seems to be less closely associated with the xylem than the virus of Pierce's disease.

As was suggested elsewhere by the present writer (Esau, 1948*b*), the classification of viruses into those occurring in both phloem and parenchyma and those rather restricted to parenchyma or to phloem (Bennett, 1940) may not cover all types of tissue relations of viruses. This is because the association between the causal agent of Pierce's disease and the xylem appears to be as definite as that between the phloem-limited viruses and the phloem tissue.

The exact nature of the relation of Pierce's disease virus to the xylem is not known at present. Presumably it lives and multiplies in the living cells of the xylem. The commonly slow movement of the virus from the inoculated leaf into the other parts of the grape seedling (as evidenced by the late appearance of internal symptoms in the internodes below and above the inoculated leaves and by the large proportion of plants in which the virus was removed with the sample taken soon after inoculation) may have been taking place through the same type of cells. However, the instances of the rapid spread of the virus from the inoculated leaves into the bases of the grapevine seedlings (tables 6 and 7), and from the bases to the tops of the alfalfa plants (Houston *et al.*, 1947) suggest the possibility of occasional movement in tracheary elements. Perhaps the virus is placed by the vector directly into the water-conducting cells—these elements are punctured by the insect during feeding—and if the xylem contents happen to be under tension at this moment, the virus could be drawn rapidly into plant parts rather distant from the place of inoculation.

The relation of the phony disease virus to the xylem is not yet clear. Bennett (1940) places this virus in the parenchyma-limited group, suggesting that it might move and multiply in the parenchymatous elements of the xylem. No definite information is available to explain why the root should be a more favorable habitat for phony disease virus than the stem (Hutchins, 1933, 1939).

The present study revealed no peculiarities in the morphology of the root, as contrasted with that of the stem, which could be used as a satisfactory explanation of the unusual distribution of the virus. It is commonly known that the ratio of living cells to dead cells in the underground organs is higher than in the aerial axes (Liese, 1924; Riedl, 1937; Beakbane, 1941). The peach roots also show more xylem-parenchyma cells and wider and higher rays than stems of similar age and diameter, but the smaller amount of suitable cells does not seem to explain the heretofore reported inability of the virus to make a successful invasion of the aerial parts. If the virus were moving only in the xylem parenchyma and rays, the morphologic and physiologic continuity of the xylem of stem and root and the thorough interconnection among the living cells of the xylem—as revealed by carbohydrate movement, for example—would seem to provide a path along which the virus could enter the stem from the root.

Fulton (1941), however, observed that certain mosaic viruses, which were introduced into roots, caused no infection of the aerial parts of the same plants. The upward movement of these viruses in the roots themselves was very slow, while the downward movement was relatively rapid. Fulton also found that viruses, which were extracted from roots, differed, in certain of their properties, from leaf extracts of the same viruses.

Bennett (1940) has suggested that a combination of a slow movement of the virus and a susceptibility to high temperatures might be the possible cause of the concentration of phony disease virus in the root; no better hypothesis has yet been offered in the literature to explain this phenomenon.

SUMMARY

Naturally infected field-grown material and artificially inoculated greenhouse plants served for the study of the anatomic effects of Pierce's disease of the grapevine and of dwarf disease of alfalfa, both of which are caused by the same virus.

The two diseases induce gum development in the xylem. The gum is deposited in vessels and in other xylem cells. In addition, the grapevine shows a precocious and excessive development of tyloses in the wood.

The xylem symptoms in these diseases appear to be primary in nature. Developmental studies on grape seedlings and on alfalfa cuttings have shown that occlusion of vessels with gum occurs before the development of external symptoms and becomes more and more pronounced with the passing of time after the inoculation. Moreover, in the grape seedling this abnormality appears first of all in the inoculated leaf.

The irregularity in cork formation in the axes and the consequent excessive accumulation of nonfunctioning phloem, which are often found in grapevines affected with Pierce's disease, might be secondary symptoms resulting from the initial disturbance in the water-conducting tissue.

The anatomic changes in affected plants, considered together with the external symptoms and the method of transmission of Pierce's disease virus, suggest a close association of this virus with the xylem tissue.

Peach roots affected with phony disease were obtained from trees growing under field conditions in Texas. All root samples showed gummosis in the

xylem. There was deposition of gum in vessels and other xylem cells and sometimes a formation of gum pockets. Thus, the internal symptoms of phony peach disease resemble those induced by Pierce's disease virus.

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PLATES



Plate 1.—Transverse sections of grape leaves from a healthy vine (*A*), and from one affected with Pierce's disease (*B*). In both views, the palisade parenchyma appears above, the spongy parenchyma below in the section. In *B*, the intercellular spaces, including the substomatal chambers (above *b*), are filled with a gumlike deposit, and the xylem elements at *d* contain gum. Details are: *a*, uppermost layer of spongy parenchyma; *b*, stomata; *c*, parenchymatous bundle extension; *d*, xylem containing gum. (Both $\times 290$.)

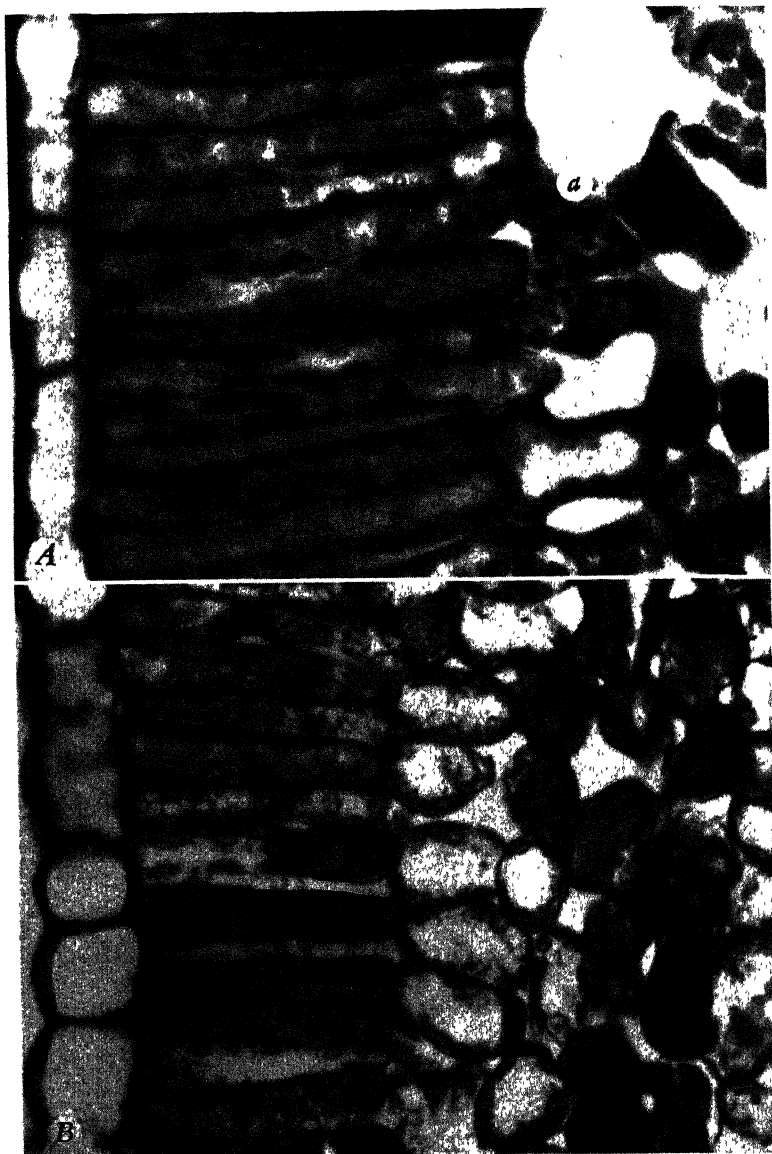


Plate 2.—Transverse sections of grape leaves from a healthy (*A*), and from a diseased (*B*) vine. In both views, the palisade cells appear to the left in the photograph. The mesophyll of the leaf in *B* differs strikingly from that in *A* in having large conspicuous starch grains in the chloroplasts. The diseased leaf also shows somewhat shorter palisade cells than the healthy. Details are: *a*, uppermost layer of spongy parenchyma. (Both $\times 750$.)

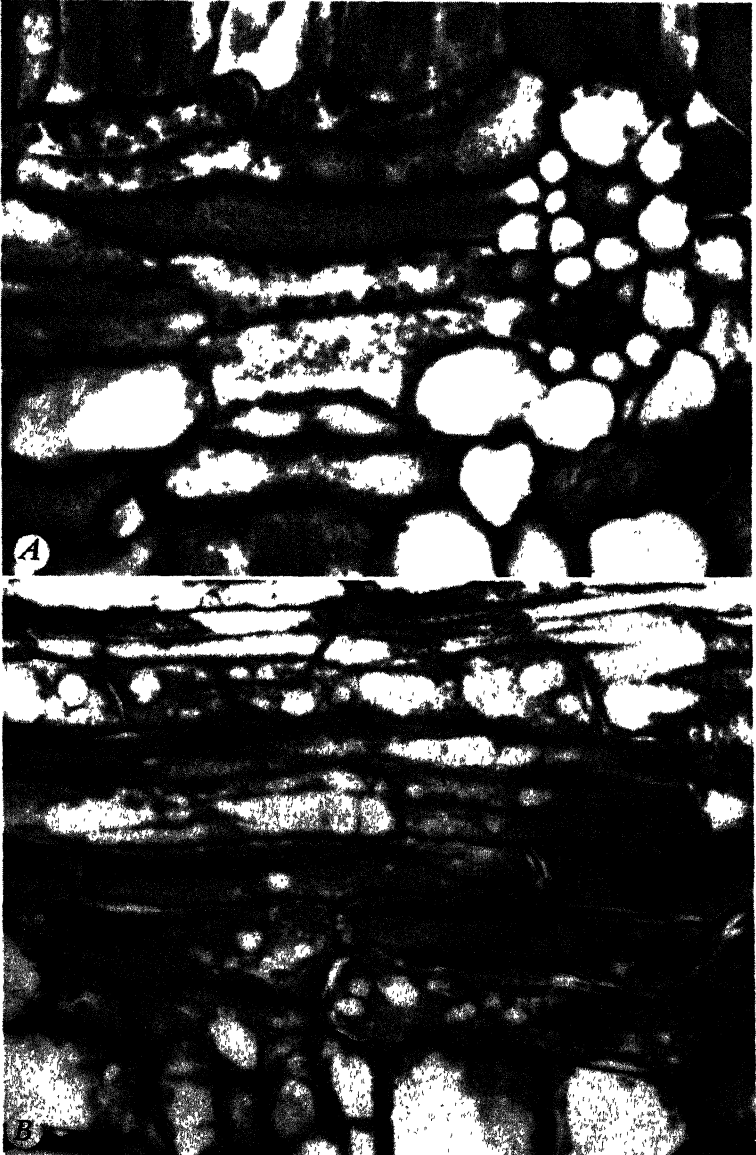


Plate 3.—Transverse sections cut parallel to vascular bundles from a healthy (*A*), and from a diseased (*B*) grape leaf. Both photographs illustrate border parenchyma of the bundles in longitudinal view. This parenchyma shows excessive accumulation of starch in the diseased leaf. (Both $\times 750$.)

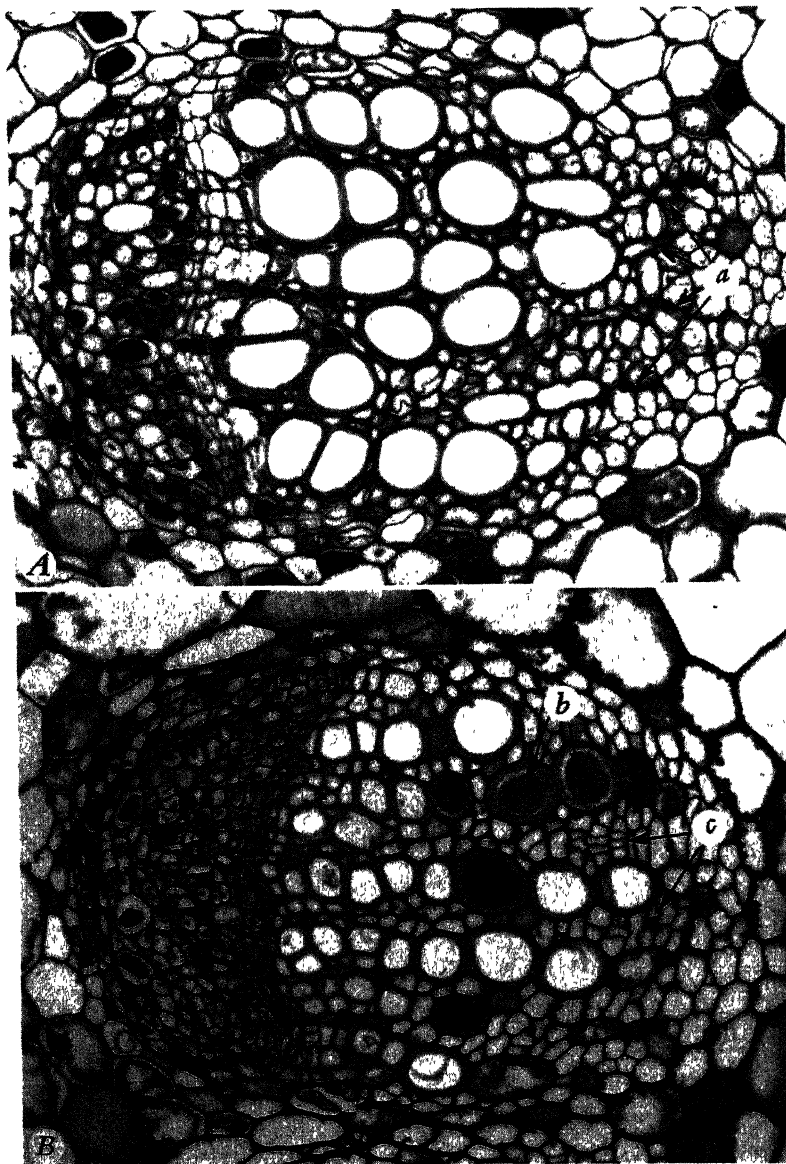


Plate 4.—Transverse sections of vascular bundles of petioles from a healthy grapevine (*A*), and from one affected with Pierce's disease (*B*). In both views, the phloem appears to the left, the xylem to the right in the section. Details are: *a*, obliterated protoxylem elements; *b*, vessels filled with gum; *c*, cell division in xylem parenchyma. (Both $\times 290$.)

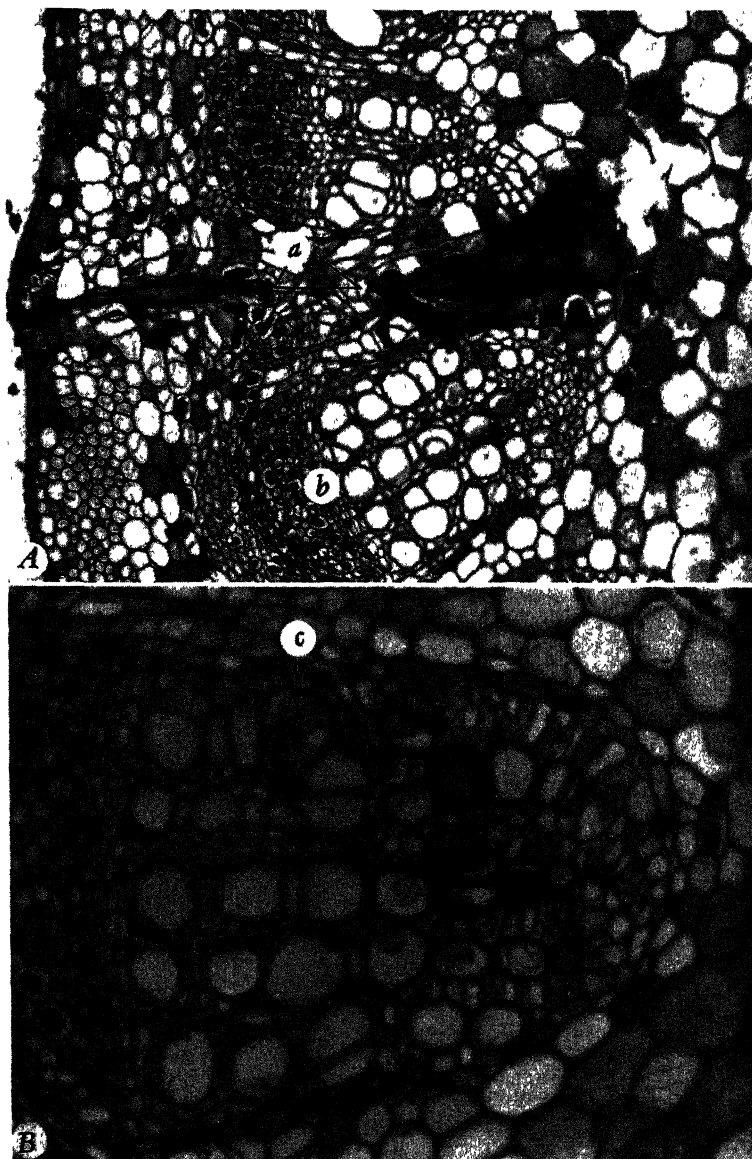


Plate 5.—Transverse sections of a shoot of grapevine sampled 14 days after it was exposed to vectors carrying Pierce's disease virus. An old feeding puncture is visible in *A*. In the cambial region at *a*, the feeding puncture has been healed over. The bundle in *B* is a continuation of the bundle *b* in *A*, taken 750 microns distant from the feeding puncture. It shows vessels with gum in the protoxylem region and a beginning of tylose formation at *c*. (*A*, $\times 140$; *B*, $\times 290$.)

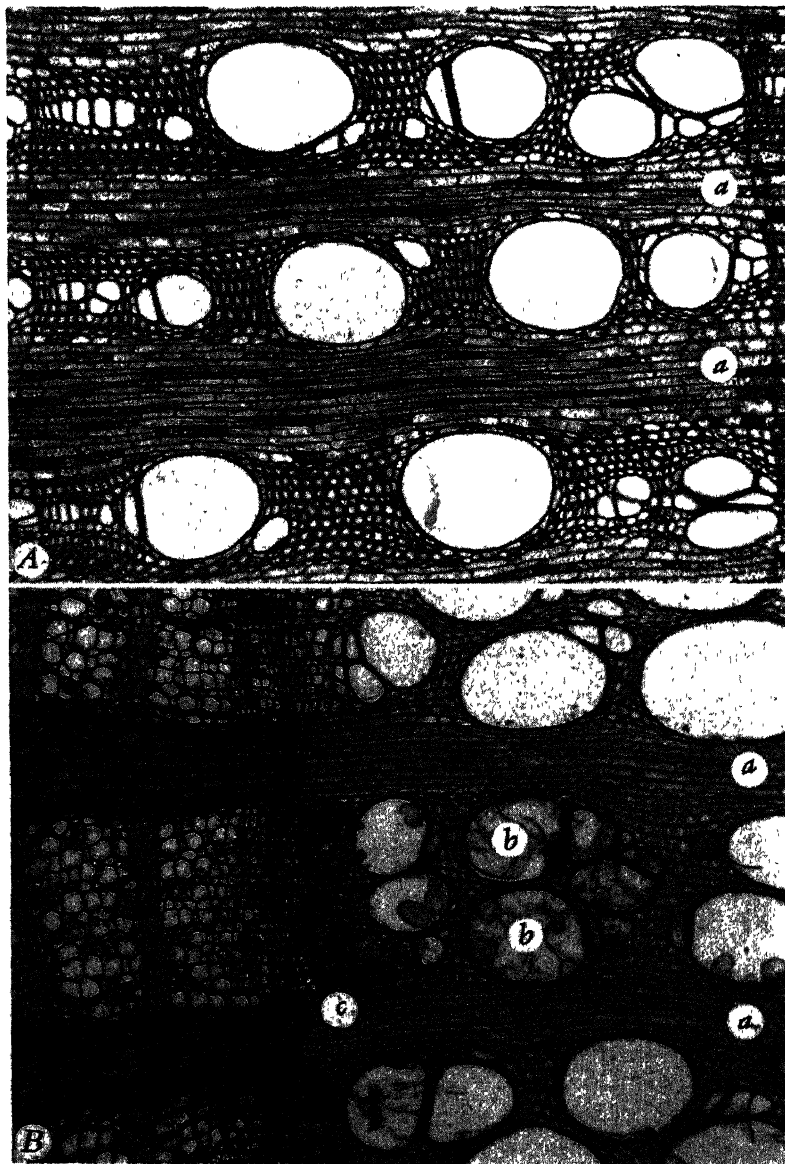


Plate 6.—*A*, Transverse section through the trunk xylem of a healthy grapevine. *B*, Transverse section through the xylem (to the right) and the phloem (to the left) of a cane from a grapevine affected with Pierce's disease. Details are: *a*, vascular rays; *b*, vessels with tyloses; *c*, group of xylem elements (located above the letter *c*) containing gum. (*A*, $\times 80$; *B*, $\times 90$.)

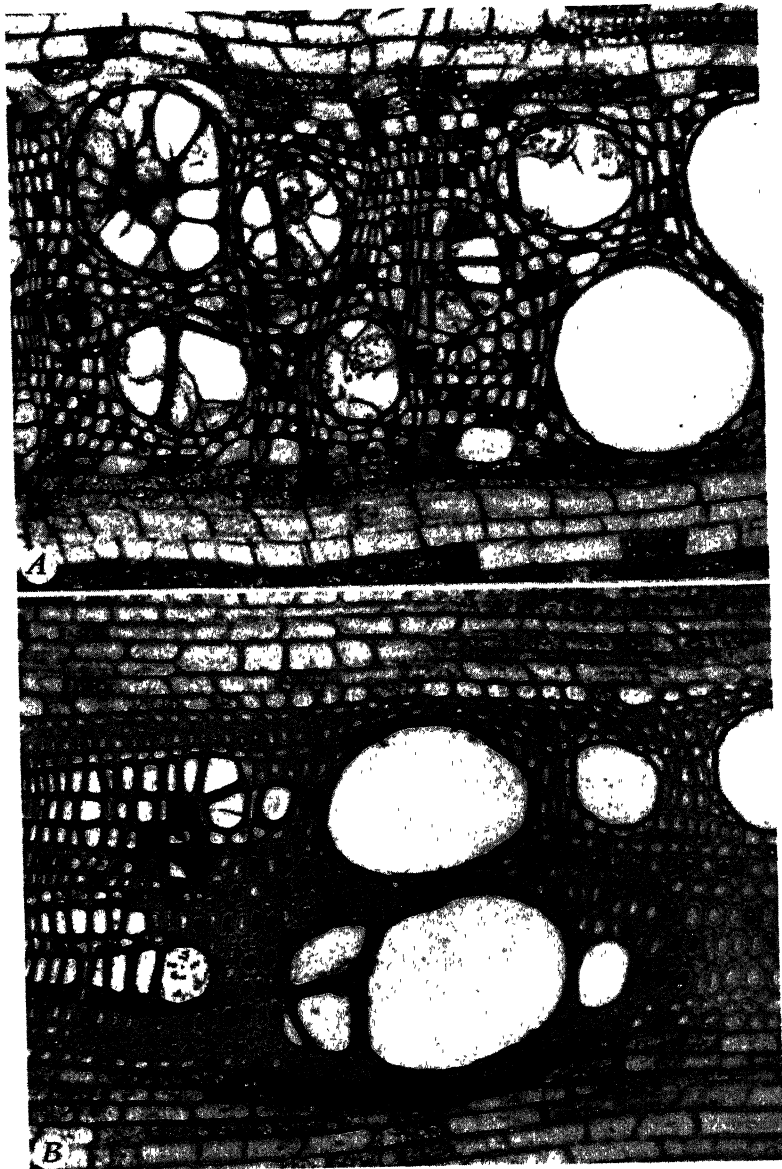


Plate 7.—Transverse sections through a rootstock (*A*), and a cane (*B*) from grapevines affected with Pierce's disease. *A* shows tyloses in the vessels to the left, and *B* illustrates accumulation of gum in various xylem cells including some ray cells (below the large vessels). (Both $\times 150$.)

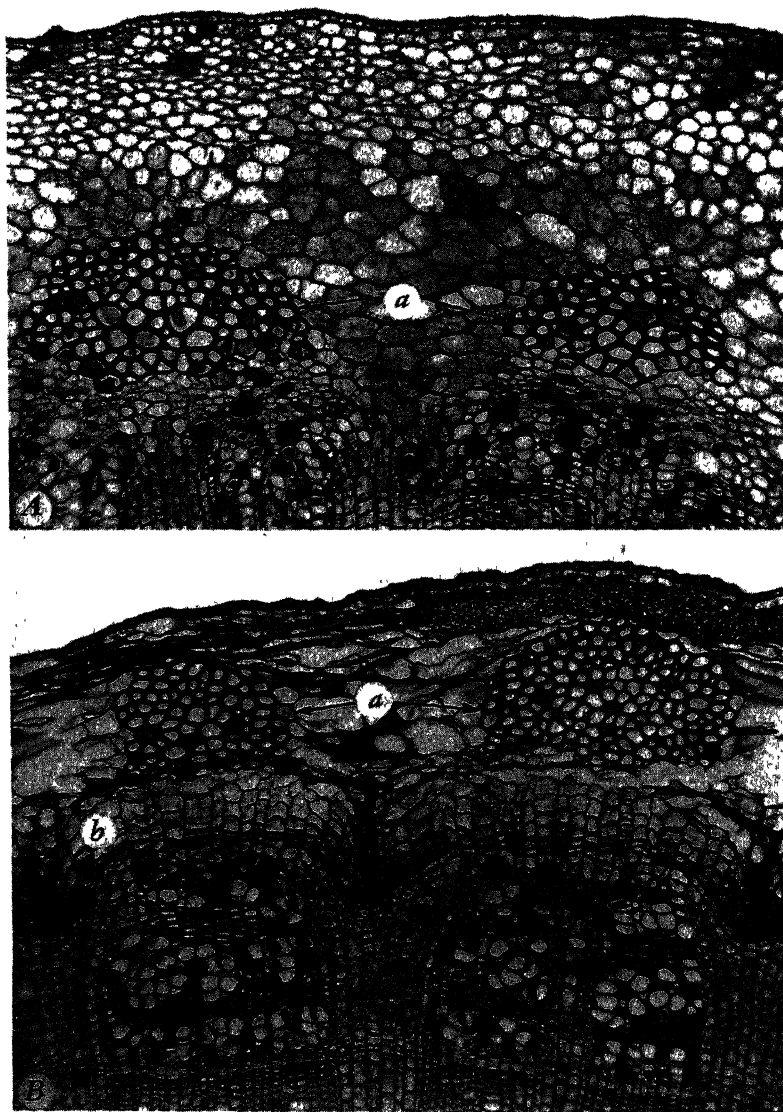


Plate 8.—Transverse sections through grapevine canes. The cortex is still attached to the cane from a vine affected with Pierce's disease (*A*), but is separated by cork from the healthy cane (*B*). Details are: *a*, primary phloem fibers; *b*, cork. (Both $\times 90$.)

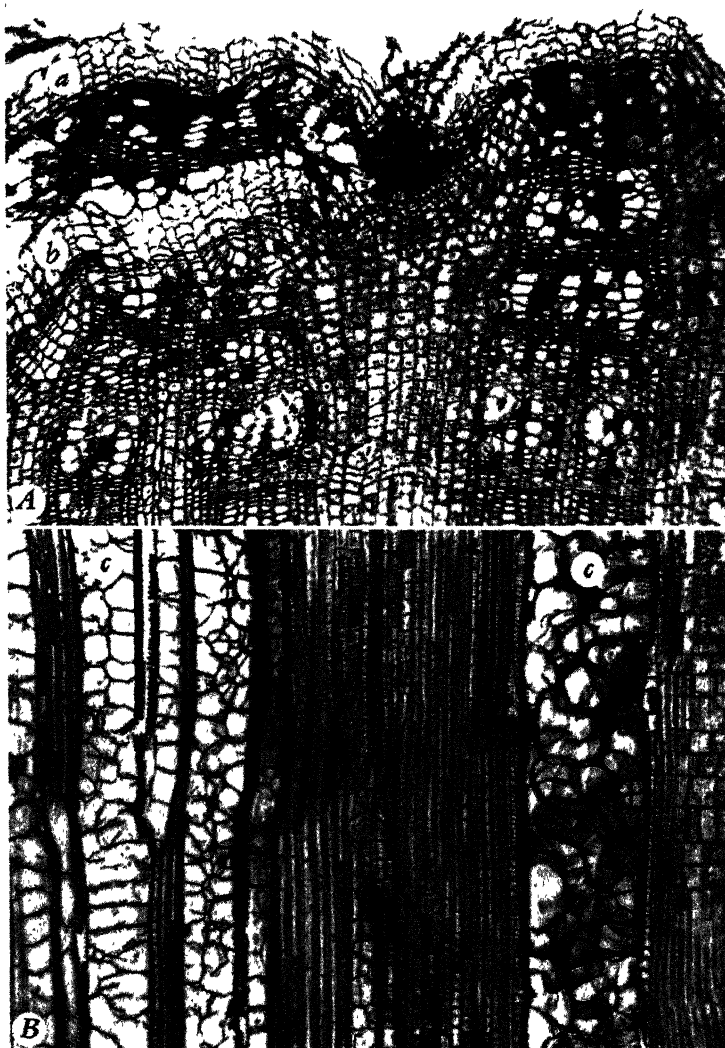


Plate 9.—*A*, Transverse section through a cane in second year of growth from a vine affected with Pierce's disease. At *a* is the cork formed in 1945. The new cork, formed in 1946 (at *b*), does not extend through the whole section, and joins the 1945 cork to the right in the figure. *B*, longitudinal section through the trunk xylem of a diseased grapevine showing vessels filled with tyloses at *c*. (Both $\times 90$.)

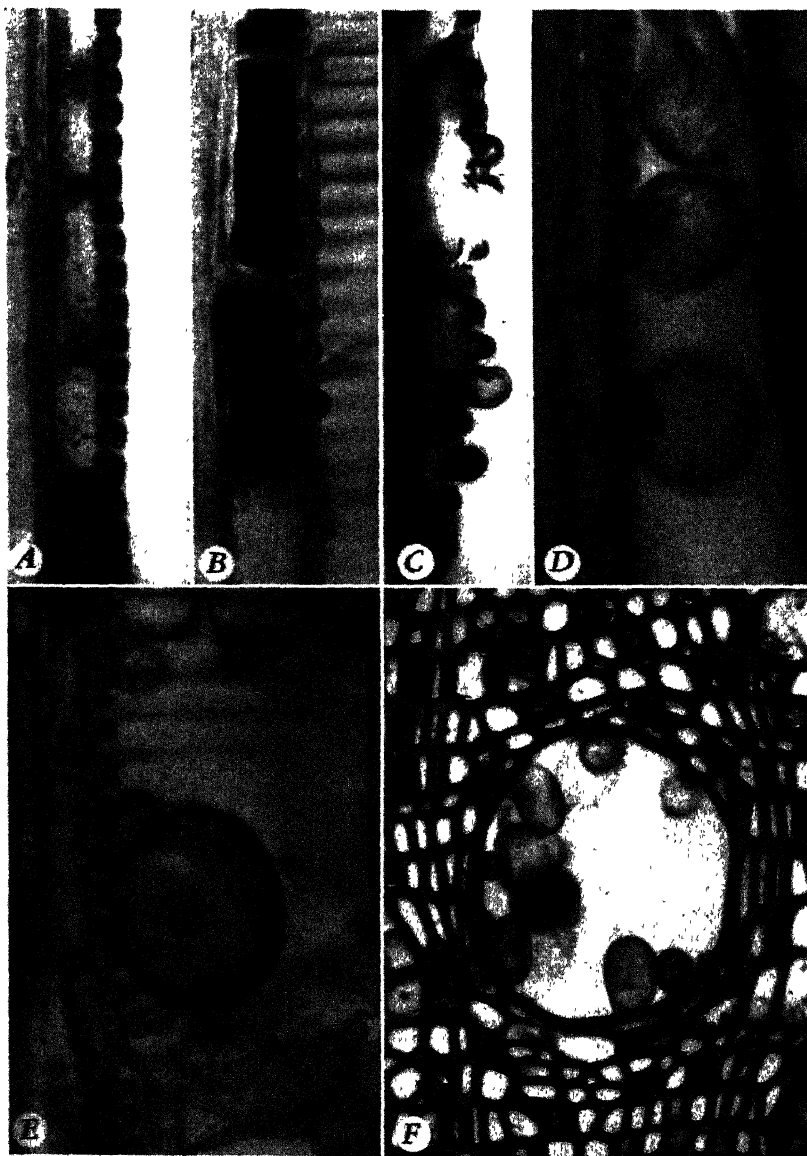


Plate 10.—Tylose development in longitudinal (*A-E*) and transverse (*F*) sections through the xylem of grapevines affected with Pierce's disease. *A*, Xylem-parenchyma cells showing, to the right, the pitting in a wall located between the parenchyma cells and a vessel. *B*, Tannin-containing xylem parenchyma cells in similar position as the cells in *A*. In *B*, the pit membranes are bulging slightly into the vessels. *C*, Similar cells as in *B*, but with somewhat more strongly extended pit-closing membranes. *D*, Young tyloses (rounded cells with nuclei) within the lumen of a vessel, and connected to the parenchyma cells from which they were derived. *E*, Two tyloses formed by the same parenchyma cell from adjacent pit membranes. *F*, Tannin-containing and tannin-free tyloses invading the same vessel. (*A-E*, $\times 750$; *F*, $\times 150$.)

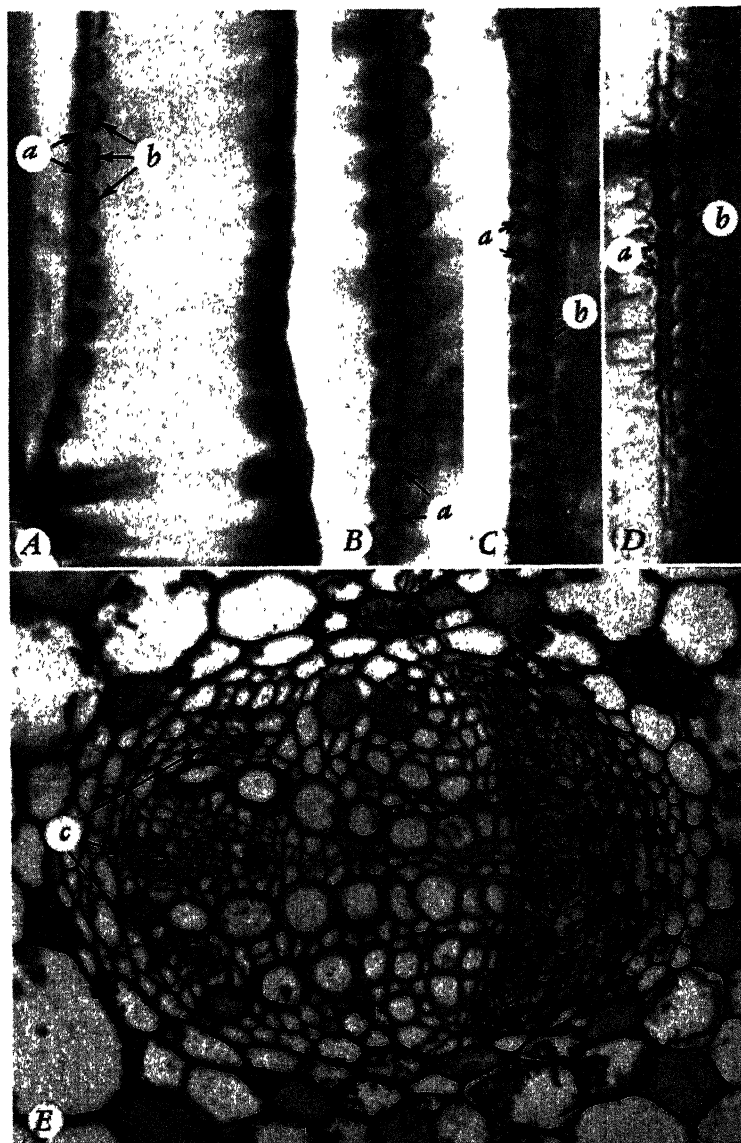


Plate 11.—*A–D*, Vessel walls from a root of alfalfa affected with the dwarf disease. Normal, thin pit-closing membranes appear in unaffected vessels in *A* and *B*. The walls from gummied vessels in *C* and *D* show thickened pit membranes. *E*, Transverse section of a petiolar vascular bundle from a grapevine affected with Pierce's disease. The phloem appears to the right. Details are: *a*, pit-closing membranes; *b*, secondary thickenings of vessel walls; and *c*, vascular bundles that arose within the xylem parenchyma. (*A–D*, $\times 1200$; *E*, $\times 290$.)



Plate 12.—Transverse sections through a root of a peach affected with phony disease. The area showing gummosis in *A* sharply contrasts with the gum-free area in *B*. Vessels, tracheids, and ray cells (*a*) contain gum in *A*. In contrast to the ray cells (*a*) in *B*, those in *A* show a more or less thorough depletion of starch. (Both $\times 180$.)

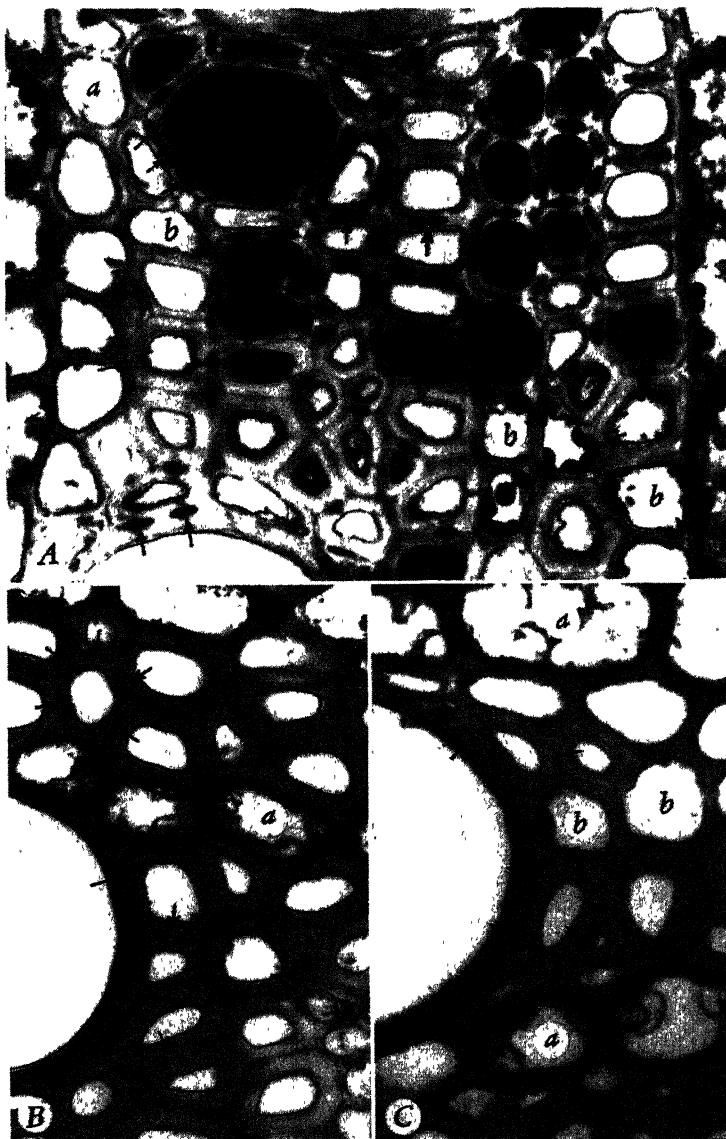


Plate 13.—Transverse sections through a root of a peach affected with phony disease. *A* shows accumulation of gum in many cells and a discoloration and increase in thickness of the pit-closing membranes, some of which are marked by arrows. In *B* and *C* appear sections free of gum and normal, thin pit-closing membranes. Details are: *a*, ray cells; *b*, xylem parenchyma cells. (All $\times 750$.)

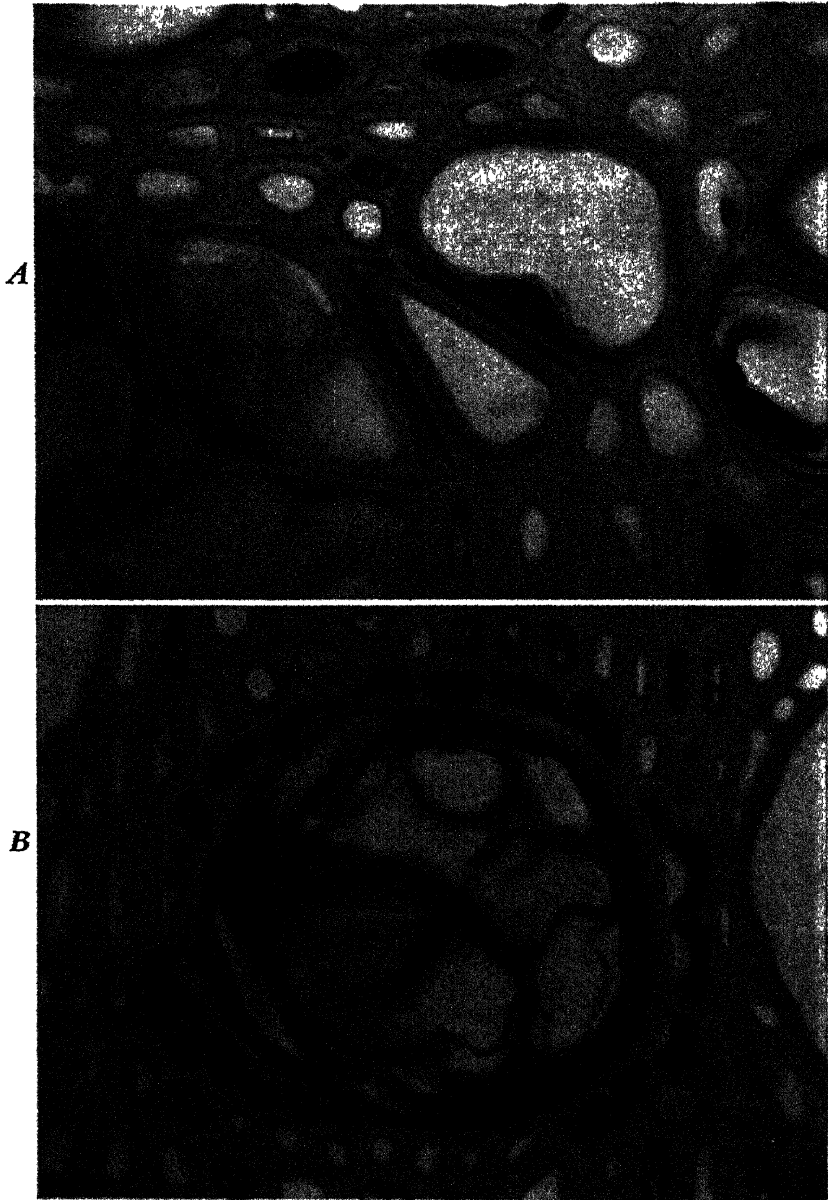


Plate 14.—Transverse sections through the xylem of grapevines affected with Pierce's disease. *A*, Several vessels contain small amounts of gum, and show thickened and deeply colored pit-closing membranes. The walls of tyloses, which are present in these vessels, are closely appressed to the vessel walls and make the latter appear double. *B*, Vessel containing gum and tyloses and surrounded by xylem-parenchyma cells, which are mostly filled with gum. In some of these cells, the gum may be distinguished from the protoplasts. Staining with Bismarck brown and iodine green. (*A*, $\times 750$; *B*, $\times 720$.)

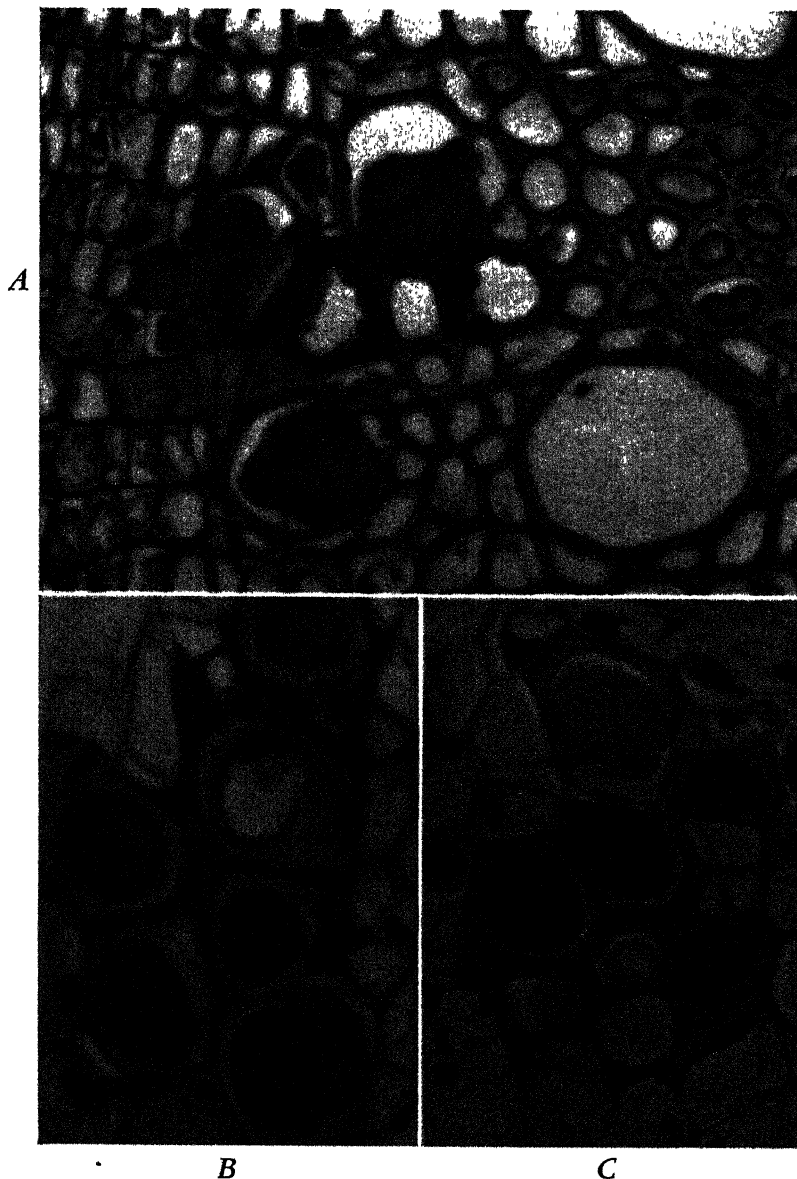


Plate 15.—Transverse sections through xylem of stem (*A*) and root (*B* and *C*) of alfalfa affected with the dwarf disease. *A*, Section stained with safranine and fast green shows at right, below, an unaffected vessel. Other vessels have variable amounts of gum variously stained. The gum, stained red, lines the vessel walls. The latter show thickened pit-closing membranes. *B* and *C*, Sections stained with acid fuchsin. The gum and the swollen pit-closing membranes (very clear in *B*) are deeply stained. Partial dissolution of the primary vessel-parenchyma wall is shown in *C* at right below. (All $\times 750$.)

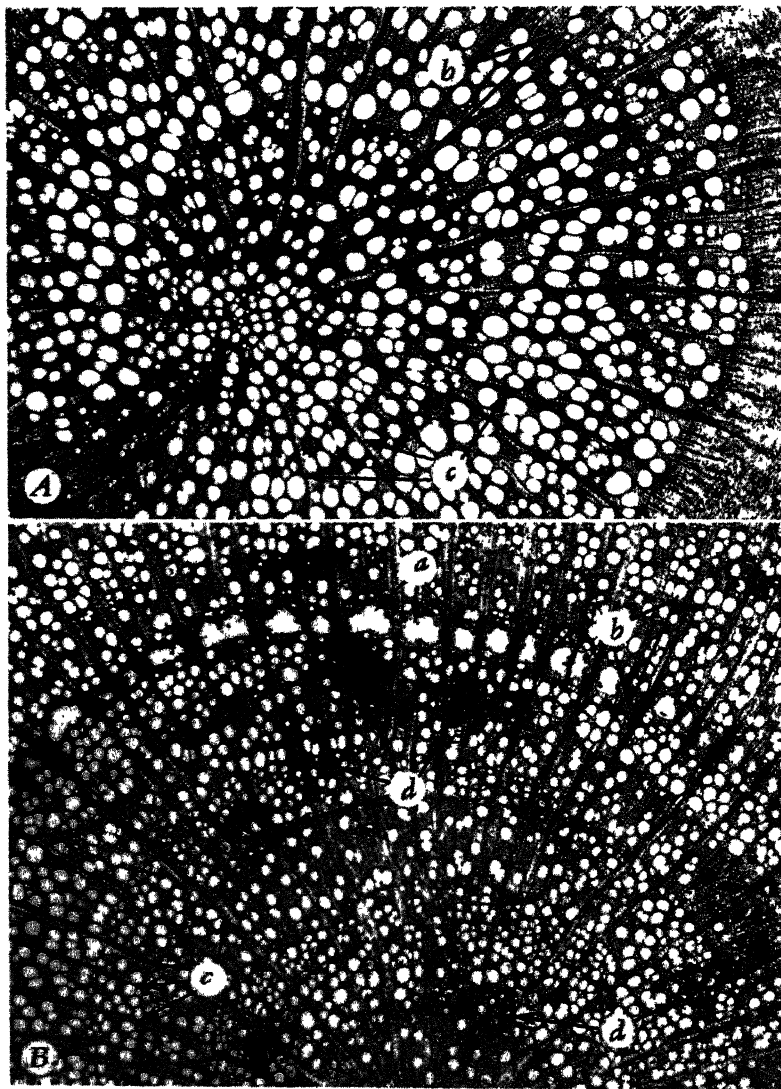


Plate 16.—Transverse sections of roots from a healthy (A) and from a phony-diseased (B) peach tree. Details are: a, gum pockets in the oldest part of an annual ring; b, one annual increment of xylem; c, xylem rays; d, areas containing vessels plugged with gum. (Both $\times 52$.)

SUGAR-BEET MOSAIC¹

HENRY H. P. SEVERIN² and ROGER M. DRAKE³

SUMMARY

Sugar-beet mosaic investigations conducted in California include tests on host range, symptomatology, properties of the virus, and various aspects of transmission by insects, especially aphids.

The economic plants in one family demonstrated to be naturally infected with the sugar-beet-mosaic virus were as follows:

Chenopodiaceae:

Beta vulgaris, sugar beet, mangel or stock beet, and garden beet

Beta vulgaris var. *cicla*, Swiss chard

Spinacia oleracea, spinach

In addition to the economic plants naturally infected, the following plants in three families were experimentally infected with the virus:

Chenopodiaceae:

Kochia scoparia var. *trichophila*, common summer cypress

Aizoaceae:

Tetragonia expansa, New Zealand spinach

Solanaceae:

Nicotiana tabacum, tobacco (Havana-type variety and Primus variety)

The sequence of symptoms on these host plants, and even on a single host plant, vary widely. The incubation period of the disease in sugar beets averages about 8 days in the greenhouse and 25 days outdoors.

Ten species of plants in five families were found to be nonsusceptible. An attempt was made to recover the virus from all plants that failed to show symptoms.

The properties of the virus extract from the leaves are summarized as follows: thermal inactivation was 60° C in 10-minute exposures; freezing the expressed juice at -18° C resulted in a monthly decrease in the number of infections to zero at the end of five months; tolerance to dilution of extracted juice was 1:5,000; and tolerance to aging *in vitro* at room temperature was 6 days.

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No transmission was obtained with eight species of insects other than aphids.

The following four aphid species which multiplied on sugar beets transmitted the virus: erigeron root aphid, *Aphis middletonii* (Thomas); bean or dock aphid, *A. rumicis* Linnaeus; pea aphid, *Macrosiphum pisi* (Kaltenbach); and green peach aphid, *Myzus persicae* (Sulzer).

The following eleven aphid species reared on other host plants are vectors of the virus: celery leaf aphid, *Aphis apigraveolens* Essig; celery aphid, *A. apii* Theobald; rusty-banded aphid, *A. ferruginea-striata* Essig; cotton or melon aphid, *A. gossypii* Glover; bur clover or cowpea aphid, *A. medicaginis* Koch; green apple aphid, *A. pomi* De Geer; cabbage aphid, *Brevicoryne brassicae* (Linnaeus); yellow willow aphid, *Cavariella capreae* (Fabricius); foxglove aphid, *Myzus solani* (Kaltenbach); honeysuckle aphid, *Rhopalosiphum comii* (Davidson); and turnip or false cabbage aphid, *R. pseudobrassicae* (Davis).

A summary of the percentages of infections obtained with all aphid species is given in table 8 (page 513).

Virus transmission by lots of 20 *Aphis middletonii*, *Macrosiphum pisi*, and *Myzus persicae* reared on mosaic beets was compared with that by mechanical inoculation. Infections obtained with the three aphid species were 20, 60, and 56 per cent, respectively, as compared with 96 per cent by mechanical inoculation of the virus extract from the plants on which they were reared. The transmission of the virus by ten aphid species reared on other host plants varied from 8 to 76 per cent, as compared with 88 to 100 per cent by mechanical inoculation of juice expressed from the same mosaic beets on which the aphids were forced to feed.

With *Myzus persicae*, the percentages of infections produced increased with the number of aphids per plant.

Short feeding time of winged aphids on mosaic and healthy beets may be of significance in the natural spread of the disease, since lots of 1, 2, 3, 4, and 5 green peach aphids gave infections averaging 0, 25, 25, 40, and 45 per cent, respectively, after having fed 5 minutes on mosaic and 5 minutes on healthy beets.

The retention of the virus by lots of 20 infective aphids varied from 1 to 3 hours under greenhouse conditions.

In one instance, aphids recovered the virus from a sugar beet infected with the virus 1 day before symptoms of the disease developed, in another instance on the same day after the first symptom appeared, and in others 1 to 2 days after the earliest symptom developed.

No infections were obtained by inoculating the cornicle exudate from infective aphids into healthy beet seedlings.

Multiple viruses in a sugar beet were separated by previously noninfective *Myzus persicae*, which recovered the sugar-beet-mosaic virus, and by previously noninfective beet leafhoppers (*Eutettix tenellus*), which recovered the curly-top virus.

INTRODUCTION

Sugar-beet mosaic is not a killing virus of sugar beets, mangels, or garden beets, and has nowhere proved to be important in commercial fields in California. During the spring of 1927, the green peach aphid, *Myzus persicae* (Sulzer), was extremely abundant on the plains and foothills in the middle

San Joaquin Valley and destroyed most of the pasture vegetation during March on the plains and foothills. After the pasture vegetation began to wilt, enormous flights of aphids occurred into the cultivated areas. That year most of the sugar beets showed symptoms of beet mosaic. Small beets were temporarily stunted, but as the season advanced, they recovered and produced a marketable crop. No information is at hand on the reduction in yield and sugar content.

On the other hand, in sugar and garden beets grown for seed, mosaic is a serious disease in California: when the stecklings or mother beets are infected before transplanting, considerable reduction in seed yield results.

An enormous amount of literature has been published on this disease in Europe and America. Papers that concern the aspects of the disease that were included in this investigation are reviewed in the following section.

An investigation was undertaken on naturally and experimentally infected host plants of sugar-beet mosaic and the sequence of symptoms was studied. Experiments were conducted to determine some of the properties of the virus. Attempts were made to transmit the virus with insects exclusive of the Aphididae, and also with aphid species that were reared on sugar beets and on other host plants. Aphids were compared with mechanical inoculation as a means of transmitting the virus. Other aspects of aphid transmission of the virus of sugar-beet mosaic discussed in this paper, include a comparison of the transmission of the virus by varying numbers of aphids, the transmission of the virus in short feeding time, the retention of the virus by aphids, and loss and recovery of the infectivity by aphids on inoculated plants. An attempt was made to separate multiple viruses in a sugar beet.

REVIEW OF LITERATURE

Common Names and Symptoms of the Disease. The first mention of this disease has been credited to Prillieux and Delacroix (1898),^{*} who called it "jaunisse," or yellows. They give the following sequence of symptoms: At first the leaves lose their normal turgescence, the petioles become less rigid, and the tip of the leaf turns down. At the same time the leaves become finely variegated, green and white. With the progress of the disease, the discolored spots coalesce; at this time the color varies from yellow to gray and the leaf becomes dry. When the plants are severely affected, the beet roots do not increase in size, although they retain their normal sugar content.

The diseases called "jaunisse" by Prillieux and Delacroix (1898) and "gulsoet" by Rostrup (1904) and Eriksson (1912) are described as beginning with a slight wilting. As Quanjer (1936) points out, this is not a symptom of sugar-beet mosaic; but he infers from the later symptoms—yellowing of the full-grown leaves and mottling of the heart leaves—that both virus yellows and mosaic must have been present.

The mottling and the fusion of the discolored spots are symptoms of a beet mosaic, but all other symptoms described are not typical of the disease as it occurs in California.

Townsend (1915) suggested the name "sugar beet mosaic" when he described the symptoms of the disease on sugar beets in the United States and stated that it was observed more than a dozen years before its publication.

^{*} See "Literature Cited" for citations, referred to in the text by author and date.

The Question of Multiple Viruses. The question of whether or not the symptoms described for beet mosaic are caused by a single virus or by multiple viruses in the same plant has been disputed by several investigators. Quanjer (1936) is of the opinion that a virus complex of mosaic and a disease of the type of virus yellows occurs in North America. This opinion is based on his contention that, contrary to Robbins (1921) and Verplancke (1934), phloem necrosis and starch accumulation are associated not with mosaic, but with virus yellows. He likewise asserts that the disease called "jaunisse" by Prillieux and Delacroix (1898) in France, and "gulsot" by Rostrop (1904) and Eriksson (1912) in Sweden, and "sugar-beet mosaic" by Townsend (1915) in the United States is a mixture of the two viruses; and that the mosaic disease investigated by Brandenburg (1927) and Böning (1927a) in Germany was not free from virus yellows in its later stages.

In another paper, Böning (1927b) used the terms "stipple, spot, point, net, and mosaic" to describe different types of the disease; but whether these are caused by different viruses is not yet decided, according to Quanjer (1936). Hoggan (1933) and Roland (1936) state that the same virus causes different types of symptoms in different leaves of the same plant. Verplancke (1933) described "speckled, veined, marbled, and pocked" types of mosaic.

In view of the various symptoms which develop with beet mosaic, Muraviov (1930) concluded it is possible that a virus complex is involved.

According to Smith (1934), "There is no evidence that more than one virus is concerned in the production of beet mosaic, and it is quite probable that the slightly different symptom expressions exhibited are due to the same agent."

After performing extensive inoculation experiments, Petherbridge and Stirrup (1935) concluded that the four types of symptoms which they studied are merely different aspects of one mosaic disease and are caused by a single virus.

It seems most unlikely that a yellows virus is involved in California or elsewhere in the United States. Sugar-beet-yellows virus has not been found in California or in any other sugar-beet district in this country. A disease called sugar-beet yellows (fusarium wilt) in the United States is caused by the soil fungus *Fusarium oxysporum* f. *betae* (Stewart) Syn. and Han.

Sugar-beet mosaic and curly top are sometimes found in the same plant in California, and some confusion in symptoms may result; the two viruses may be separated by the use of insect vectors (Severin, 1929). Another method of separating them is reported in a later section of this paper.

Classification of the Virus. Johnson and Hoggan (1935) in their key for plant viruses gave the chief diagnostic features of the sugar-beet-mosaic virus based on modes of transmission, properties of virus, and distinctive or specific symptoms.

Smith (1937) classifies the sugar-beet-mosaic virus as *Beta virus 2* Lind, and lists the following synonyms: beet-yellows virus, Prillieux and Delacroix (1898); beet-mosaic virus, Lind (1915); sugar-beet virus 2, Johnson and Hoggan (1935); mosaic of sugar beet, Smith (1933).

Holmes (1939) classifies the sugar-beet-mosaic virus as *Marmor betae* in the family *Marmoraceae* and gives the following synonyms: beet-jaunisse virus, beet-yellows virus, beet-mosaic virus, sugar-beet virus 2, *Beta virus 2*.

McKinney (1944) established the "Genus 3. Poccile, gen. nov. as a synonym of *Marmor*, Holmes (1939) P.P."

Papers on properties of the virus are reviewed in connection with the work done on that aspect in the present investigation (page 497).

Distribution of the Disease. Prillieux and Delacroix (1898) observed the diseased beets in northern France in the vicinity of Paris in 1896. The distribution of beet mosaic, apart from the accidental presence of virus yellows in the same plants described by some investigators, on varieties of beets has been reported from Europe and Japan as follows:

Belgium: Verplancke (1933, 1934, 1934-35), De Haan and Roland (1935), Roland (1936)

Bohemia: Uzel, according to Molz (1926)

Denmark: Lind (1915)

England: Smith (1934, 1937), Petherbridge and Stirrup (1935), Ogilvie (1942), Moore (1943)

France: Lind (1915), Ducomet (1928, 1929)

Germany: Lind (1915), Molz (1926), Böning (1927*a, b, c*), Schmidt (1927, 1935)

Holland: Van der Meulen (1928), De Haan and Roland (1935), Quanjer (1936), Quanjer and Roland (1936), Roland (1936)

Russia: Proida (1930), Boryssewicz (1930), Muraviov (1930), Novinenko (1930), Shevtshenko (1930)

Sweden: Lind (1915), Eriksson (1912)

Japan: Hino (1933)

Conners (1935) was first to report mosaic on mangels in Canada as a disease new to that country. He also found a trace of a mosaic disease at Saskatoon on Swiss chard.

Townsend (1915) was the first to describe the symptoms of sugar-beet mosaic occurring in the middle and western portions of the United States. Reports from observers indicate that the disease on sugar beets, garden beets, and Swiss chard has become increasingly prevalent in this country. The distribution of sugar-beet mosaic as noted by those who have conducted specific investigations and by others who have conducted surveys of plant disease in various states in connection with the United States Bureau of Plant Industry is as follows:

California: Plant Disease Reporter⁵ (1921*b*), Hoggan (1933), sent by C. W. Bennet

Colorado: Robbins (1921), Plant Disease Reporter (1921*b*, 1926, 1944)

Idaho: Hoggan (1933), sent by P. N. Annand

Indiana: Plant Disease Reporter (1921*a*,⁶ 1923)

Kansas: Plant Disease Reporter (1921*b*, 1923)

Nebraska: Robbins (1921)

New Mexico: Plant Disease Reporter (1927, 1928*b*)

Texas: Plant Disease Reporter (1929)

Utah: Plant Disease Reporter (1923*a, b*, 1936)

Washington: Plant Disease Reporter (1930*a, b*, 1936), Jones (1931)

Wyoming: Plant Disease Reporter (1944)

Economic Importance of the Disease. Considerable differences have been reported in the economic importance of the disease in various countries.

⁵ Numerous references of the occurrence of sugar-beet mosaic have appeared in the Plant Disease Reporter. They are listed in chronological order rather than under the names of the collaborators and editors in the "Appendix to Citations" at the end of the paper.

⁶ In a personal interview, Gardner, the collaborator who sent in the report from Indiana in 1921, stated that the virus disease proved later to be sugar-beet savory and not mosaic.

Prillieux and Delacroix (1898) stated that when plants are severely attacked, the roots do not increase much in size, but retain their normal sugar content; and that the total loss of the crop is about 50 per cent.

Molz (1926) estimated a reduction in yield of about 40 per cent in fields of mosaic sugar beets in Saale, Saxony.

Böning (1927*b*) estimated from field experiments at Bonn, Germany, that an average loss of 20 per cent resulted from mosaic, while the sugar content of fodder beets was reduced to one third of the normal. He also stated that development during the first year of the "curl mosaic," a severe form of the disease in which the leaves became curled and distorted, resulted the second year in stunted plants and poor seed production.

Shevtshenko (1930) calculated a reduction of 12.9 per cent in seed yield, and an average decrease in sugar content of 0.75 per cent in diseased sugar beets in the Kharkov district, U.S.S.R.

Prioda (1930) reported a maximum deficiency of sugar of 13 per cent as a result of the disease in the Kharkov district, U.S.S.R.

The economic importance of sugar-beet mosaic on beet seed plants has been discussed by five American plant pathologists. Robbins (1921) found scattered plants of sugar-beet seed plants in Colorado to be severely mottled, crumpled, twisted, and contorted, and the yield of seed reduced to a small amount. Crawford (Plant Disease Reporter, 1927; see Appendix to Citations) reported that mosaic caused considerable damage by dwarfing and stunting seed beets in New Mexico. Linford (Plant Disease Reporter, 1928*a*) mentioned that although mosaic had been known for several years in Utah, it had nowhere proved important. According to Jones (1931), growers and seedsmen contended that sugar-beet mosaic had reduced the yield of garden-beet seed at least 50 per cent on 1,200 to 1,500 acres in Skagit County, Washington, during the preceding five years. Connors (1935) estimated that about 5 per cent of the mangels grown for seed on Lulu Island near Vancouver were infected.

Soil Transmission. Robbins (1921) suggested the possibility that the virus might overwinter in the soil; but Böning (1927*a*) proved that the soil plays no part in transmission of the disease. Other writers who have discredited the possibility of soil transmission of the disease are Shevtshenko (1930), Jones (1931), and Verplancke (1933, 1934). Smith (1934) mentioned that most workers are agreed that the virus of beet mosaic is not carried in the soil.

Seed Transmission. With the exception of Ducomet (1929) and Verplancke (1933), investigators in general are not of the opinion that the virus is seed-borne. The latter reported that he had confirmed Ducomet's results by obtaining seed transmission in 7.1 per cent of the beets grown from seeds of mosaic mother beets 2 months after growth aboveground. Petherbridge and Stirrup (1935) pointed out, however, that 2 months appears to be a somewhat lengthy period for the development of the symptoms; and the possibility of accidental infection by insects or other means naturally suggests itself.

Lind (1915), Böning (1927*b*), and Van der Meulen (1928) all concluded that the virus is not seed-transmitted.

To quote Quanjer (1936): "Contrary to what Verplancke (1933) claims to have found, the disease is not seed-transmissible. He claims to have cor-

roborated Ducomet's view in this respect, but this claim is based on a misunderstanding of what Ducomet (1929) wrote."

Insect Transmission. The following species of aphids have been recorded in the literature as vectors of the sugar-beet-mosaic virus:

Green peach aphid, *Myzus persicae* (Sulzer): Robbins (1921), Van der Meulen (1928), Jones (1931), Verplancke (1933), Hoggan (1933), Smith (1934), Petherbridge and Stirrup (1935), and Roland (1936)

Black beet or bean aphid, *Aphis fabae* Scopoli: Böning (1927a, 1927b, 1927c), Schaffnit (1927), Novinenko (1930), Verplancke (1933) [*Dorsalis fabae* (Scopoli) = *A. fabae*], Smith (1934), Petherbridge and Stirrup (1935), and Ogilvie (1942)

Macrosiphum cognatus Fieber: Novinenko (1930)

Macrosiphum pelargonii (Kaltenbach): Böning (1927b), Schaffnit and Weber (1927), and Verplancke (1933)

Potato aphid, *Macrosiphum solanifolii* (Ashmead) (= *M. gei* Kaltenbach and *M. ulmariae* Shrank): Van der Meulen (1928), Hoggan (1933). Smith (1934) failed to transmit the sugar-beet-mosaic virus with this species

Pound (1947) reported that the black bean aphid, *Aphis fabae*, and the green peach aphid, *Myzus persicae*, found commonly in beet fields in the Puget Sound section, are vectors of the virus. He obtained transmission of the virus with the cabbage aphid, *Brevicoryne brassicae*, but did not think that this insect is a common vector.

Overwintering of Virus. Prillieux and Delacroix (1898) noticed that diseased beets planted for seed in the spring developed symptoms on the new leaves.

Robbins (1921) demonstrated that the virus retained its vitality in the steckling throughout the silo period and stated that this was the only means of overwintering then known.

Böning (1927a) suggested the virus overwinters in frost-resistant spinach and seed beets.

Jones (1931) reported that the disease will overwinter in the beet roots in the pits, and such infected mother-beets act as a source of infection when planted in the field the following spring.

Quanjer (1936) stated that the virus remains in the roots destined for seed production.

Host Range. A number of investigators have briefly reported sugar-beet mosaic on some hosts among economic plants.

Lind (1915) reported mosaic on garden beets in Denmark, Sweden, France, and Germany, but stated that the disease was never found on sugar beets.

According to Böning (1927a) mosaic disease of beets is widespread in Germany on all cultivated varieties of beets. In another paper Böning (1927b) reported aphid transmission of the virus from beets to spinach and vice versa. Spinach was injured much more severely than beets. He also succeeded in transferring the virus from spinach to mangels and vice versa, and suggested that the two mosaics are closely related, if not identical (Böning, 1927c).

Van der Meulen (1928) failed in all attempts on intertransmission of the virus from beets to spinach by means of aphids.

Hoggan (1933) infected Bloomsdale and Virginia Savoy spinach with the virus by mechanical inoculation and produced local symptoms on Havana-type tobacco (*Nicotiana tabacum*) by infective aphids.

Smith (1934) transmitted the virus by mechanical inoculation or aphids to sugar beets, mangels, garden beets, spinach beets, sea-kale beets (chard), and spinach, and stated that no varieties of sugar beets or mangels resistant to mosaic are known.

Petherbridge and Stirrup (1935) mentioned that infection of turnips, tobacco, spinach, and beans with the virus of sugar-beet mosaic has been proved, but no evidence was given to substantiate their statement.

De Haan and Roland (1935) stated that in Holland mosaic is found more frequently on fodder beets than on sugar beets.

Pound (1947) reported that the sugar-beet mosaic virus infects all chenopodiaceous plants by mechanical inoculation. Of the species tested in seven other families, the virus infected only *Verbena hybrida*, *Viola tricolor*, *Stellaria media*, *Tetragonia expansa*, *Aster amellus*, *Zinnia elegans*, *Amaranthus retroflexus*, *Capsella bursa-pastoris*, and *Iodanthus pinnatifidus*. The first two of these host plants were symptomless carriers.

Six weeds in the family Chenopodiaceae have been experimentally infected with mosaic (Severin and Drake, 1947).

MATERIALS AND METHODS

Source of Virus. The original sugar-beet-mosaic virus was obtained from a field of naturally infected sugar beets near San Pablo, California. Mechanical inoculation of healthy sugar beets grown under cover in the greenhouse was carried out to obtain a virus supply, and this was maintained by continuous inoculations during the experiments.

Virus Extract. In the preparation of the virus extract used in mechanical inoculations, the blades and petioles from infected plants were washed in distilled water and reduced to a pulp in a sterile mortar or food chopper. Juice was pressed from the pulp through two layers of cheesecloth into sterile containers. Methods used in determining properties of the virus are given in the section on that subject.

Mechanical Inoculation. Mechanical inoculations were performed essentially by the same method as described by Rawlins and Tompkins (1936). Cotton swabs on wooden splints were used; these were discarded and the hands were carefully washed after each trial or series of inoculations. The plants were washed with water shortly after inoculation to remove the inoculum and carborundum, and to prevent wilting.

Production of Noninfective Aphids. The green peach aphid, *Myzus persicae* (Sulzer), was used in most tests. Noninfective aphids were obtained by transferring mature, apterous aphids from populations collected in the field to favorable healthy host plants. On the following day the offspring from the mature aphids were transferred to a second healthy plant and allowed to multiply. Populations of noninfective aphids were maintained on healthy plants. No symptoms appeared on these plants. To test whether the populations remained noninfective, frequent checks were made on plants on which the noninfective aphids were reared, by removing a leaf and inoculating the extracted juice into healthy beets. The disease was not produced in any case.

Various sizes of lawn-covered insect cages with glass fronts and circular wooden bases, as described in a previous paper (Severin, 1936), were used in

confining aphid populations or lots of aphids transferred to beets during the experiments. The beets were exposed to aphids for at least 2 days, then were fumigated with Nicofume tobacco-paper insecticide, and placed in insect-proof cages for symptoms to develop.

Methods of Transferring Aphids. Transfers of noninfective or infective aphids to healthy host plants or to diseased beets were made by cutting off leaves carrying high populations and placing them on the inner leaves, whenever a new food supply was necessary. In those experiments requiring accurate counts of the number of aphids used, the insects were transferred from plant to plant with a moistened camel's-hair brush. Precautions were taken not to injure them in any way.

Segregation of Plants. Inoculated host plants were held for observation of symptoms in an insect-proof cage. Any plants on which symptoms developed were removed to another cage. If no symptoms appeared at the end of one month, the plants were discarded. Healthy plants were kept in a separate insect-proof cage.

HOST RANGE, INCUBATION PERIOD OF DISEASE, AND RECOVERY OF VIRUS

Economic Plants Naturally Infected. The following economic plants in two families were demonstrated to be naturally infected with sugar-beet mosaic in California. The virus was recovered from these host plants and transferred to sugar beets by mechanical inoculation.

Chenopodiaceae:

Beta maritima

Beta vulgaris, sugar beet, mangel or stock beet, and garden, table, or red beet

Beta vulgaris var. *ciola*, Swiss chard

Spinacia oleracea, spinach

Aizoaceae:

Tetragonia expansa, New Zealand spinach

Economic Plants Experimentally Infected. The number of species and varieties of host plants experimentally infected with sugar-beet mosaic, the incubation period of the disease, and the recovery of the virus are shown in table 1.

Nonsusceptible Economic Plants. The following economic plants, tested by mechanical inoculation, were nonsusceptible to sugar-beet mosaic. An attempt was made to recover the virus from all plants which failed to show symptoms.

Cruciferae:

Brassica oleracea var. *botrytis*, cauliflower (February variety)

Brassica oleracea var. *capitata*, cabbage (Winter Colma variety)

Mathiola incana var. *annua*, annual stock or gilliflower

Cucurbitaceae:

Cucumis sativus, cucumber (White Spine variety)

Leguminosae:

Phaseolus vulgaris, bean (Lady Washington variety)

Vicia faba, horse bean

TABLE 1
HOST RANGE OF SUGAR-BEET MOSAIC, INCUBATION PERIOD OF
DISEASE, AND RECOVERY OF VIRUS

Common and scientific name of plant, and variety	Number of plants inoculated	Plants infected	Incubation period of disease, days		Recovery of virus	
			Range	Average	Sugar beets inoculated	Sugar beets infected
Chenopodiaceae, goosefoot or saltbush family						
<i>Beta maritima</i>	10	10	9-15	10.3	10	10
Sugar beet (<i>Beta vulgaris</i>)						
Klein Wanzleben.....	5	5	6-7	6.8	5	5
A. 600.....	20	19	8-11	9.2
U.S. No. 12.....	20	20	8-11	9.0
U.S. No. 14.....	20	20	8-11	8.4
U.S. No. 33.....	20	19	8-13	12.6
U.S. No. 35.....	20	18	8-13	10.6
Mangel or stock beet (<i>Beta vulgaris</i>)						
Danish Sludstrup.....	5	5	7-9	8.2	5	5
Rose Top Giant Half Sugar.....	5	5	7-9	8.0	5	5
Golden Tankard.....	10	10	7-8	7.3	10	10
Red Eckendorf.....	5	5	6-9	7.4	5	5
Yellow Eckendorf.....	5	5	7-8	7.6	5	4
Garden beet (<i>Beta vulgaris</i>)						
Crimson King.....	5	5	6-7	6.2	5	4
Crosby's Egyptian.....	5	5	6	6.0	5	5
Dark Red Ferry's strain.....	5	5	6	6.4	5	3
Dark Red Morse's strain.....	5	5	6-7	6.4	5	5
Extra Early Flat Egyptian.....	5	5	6-8	6.6	5	5
Good for All.....	5	5	6-7	6.4	5	5
New Century.....	5	5	6-8	7.0	5	5
Swiss chard (<i>Beta vulgaris</i> var. <i>cicla</i>)						
Large-ribbed Dark Green.....	10	9	6-14	11.5	10	10
Large-ribbed White.....	20	17	7-19	11.6	20	20
Lucullus.....	10	10	6-9	7.5	5	5
Spinach (<i>Spinacia oleracea</i>)						
Giant Thick-leaved Nobel.....	10	9	8-11	9.2	10	9
Long Standing Bloomsdale.....	15	14	8-15	10.6	15	14
Prickly Seeded.....	10	10	7-11	9.3	10	10
Virginia Savoy.....	5	5	11-15	13.2	10	9
Viroflay.....	10	10	7-12	8.3	10	9
Common summer-cypress (<i>Kochia scoparia</i> var. <i>trichophila</i>).....	10	7	7-9	10	5
Aizoaceae, carpet-weed family						
New Zealand spinach (<i>Tetragonia expansa</i>).....	10	7	8-12	10.6	10	9
Solanaceae, nightshade family						
Tobacco (<i>Nicotiana tabacum</i>)						
Havana-type (local lesions).....
Primus (local lesions).....

Solanaceae:

Capsicum frutescens var. *grossum*, bell pepper (California Wonder variety)

Lycopersicon esculentum, tomato (Marglobe and Santa Clara Canner varieties).

Nicotiana glutinosa

Umbelliferae:

Apium graveolens var. *dulce*, celery (Golden Self-blanching variety)

SYMPTOMATOLOGY

Beta maritima. The sugar beet is presumably a derivative of *Beta maritima* indigenous to the Mediterranean region of Europe.

The early symptoms on *Beta maritima* are cleared veins and veinlets on the lower leaves of the apical and axillary shoots occurring simultaneously with, or closely following, stunting of the growing tips (plate 1, A). A chlorotic mottling follows rapidly after the clearing of the veinlets. The young leaves are curled downward, frequently cupped inward or outward, the margins rolled inward and often twisted (plate 1, A).

Small necrotic rings and larger necrotic areas appear on the older growing leaves, usually within 13 days after inoculation. The necrotic areas are located interveinally and some extend along the midrib or lateral veins. Sometimes a reddish discoloration of the midrib or lateral veins occurs, which later develops into necrotic areas. Necrosis of a portion or all of the leaf margins occurs and sometimes extends along the petiole to its attachment. Converging of advancing necrotic margins from opposite sides results in death of the leaf, while necrosis advancing between the lateral veins toward the midrib results in killing of the tissues on one side. Mature leaves did not become perceptibly chlorotic or necrotic. Dark streaks appear on the stem and later become sunken and necrotic (plate 1, A).

The sepals turn black and necrosis spreads to other flower parts, resulting in death of the flowers. The plants usually died within 3 or 4 weeks after inoculation.

The incubation period of the disease in the greenhouse was 9 to 15 days (table 1).

Sugar Beet. The symptoms on sugar beets are so diverse that, as noted earlier, some plant pathologists have suggested that more than one virus may be concerned in the production of the disease. It was considered advisable to present a detailed description of the sequence of symptoms observed on the leaves of sugar beets.

The first evidence of infection in nearly all cases, and one not readily discernible upon cursory observation, was the presence of a few, minute yellow or pale-green flecks (plate 2, A) on the youngest leaves, as if the juncture of two veinlets had become cleared and slightly widened (plate 2, B). This condition becomes apparent only when the leaves are held toward the light. A few hours later a definite clearing of the veinlets occurs (plate 4, A), usually spreading within 24 hours over the entire leaf (plate 4, B).

On a few occasions the first symptom to appear on the youngest leaves is small, scattered, chlorotic dots and irregular, chlorotic areas (plate 2, D), which gradually enlarge (plate 2, E) into a mottled pattern in later stages. In our observations, this was the exception, rather than the rule, contrary to the observations of Smith (1934).

Widening and merging of chlorotic areas along the cleared veinlets (plate 4, *C*) marks the beginning of an irregular blotching type of chlorosis. During or at the inception of this condition, the leaves in adjacent whorls begin to take on either a diffuse or a well-defined blotching type of chlorotic mottling. The mosaic pattern may consist of green blotches in a faintly chlorotic leaf (plate 3, *A*) or well-defined chlorotic blotches in the green portion of the leaf (plate 3, *C*). Young and older leaves rapidly become chlorotic (plates 2, *I*; 3, *B*), to such an extent that a few green areas appear in sharp contrast on a chlorotic background (plate 3, *D, F*). Young leaves often become crinkled along the margin; and sometimes large, deep-green, blisterlike elevations develop on the upper surface (plate 3, *E*), while other leaves from the same plant may not show the blisterlike elevations (plate 3, *G*).

Later, 3 weeks after inoculation, the blotching type of chlorosis was frequently replaced, particularly on the intermediate leaves, with a different pattern. This consisted for the most part of various sizes of chlorotic rings with normal green centers (plate 6, *A*); or numerous, scattered, chlorotic dots (plate 2, *C*) on the intermediate leaves of the same plant, or a mixture of both.

Unusual types of symptoms were observed occasionally on young leaves after clearing of the veinlets. In one of these, chlorosis was restricted to interveinal spaces (plate 2, *F*) and a veinbanding of normal green tissue occurs. Some leaves show but a few scattered dots or small, chlorotic areas which enlarge very slowly, if at all (plate 2, *D*). In another type a chlorotic band extends the length of the midrib and partly along some of the lateral veins (plate 2, *G*).

Under natural conditions, sugar beets in an advanced stage of the disease often show a necrosis of the midrib, veins, and petioles; and this sometimes occurs on experimentally infected plants (plate 2, *H*).

A dwarfing of the heart leaves of infected beet seedlings usually occurs; however, there was a general tendency to grow out of the stunting, and even at times to outgrow the symptoms, as previously noted by Robbins (1921). Temperature influences the incubation period and the severity of symptoms. Under greenhouse conditions the period between inoculation and appearance of cleared veinlets ranged between 6 and 13 days (table 1). On October 26, 1936, 10 beet seedlings were inoculated with juice from one mosaic beet. Five of these were kept in the greenhouse, and the remaining 5 were placed in screened cages outdoors. The average time required for symptoms to develop on the beets in the greenhouse was 7.8 days. Mottling appeared on all the leaves within a week after symptoms first appeared. The 5 plants kept outdoors required an average of 25.2 days for symptoms to develop, and chlorosis was confined to the inner whorl of leaves. Two weeks after symptoms appeared, these plants were brought in the greenhouse, and symptoms appeared on the outer leaves within 5 days.

Mangels, or Stock Beets. The foliage symptoms of sugar-beet mosaic on varieties of mangels, or stock beets, were essentially the same as those on sugar beets; hence they will not be described.

Attempts to infect five varieties of mangels by mechanical inoculation were successful in 100 per cent of the trials (table 1). The incubation period of the disease in the greenhouse was 6 to 9 days (table 1).

Garden, Table, or Red Beets. Seven varieties of garden, table, or red beets were inoculated with the sugar-beet-mosaic virus, and no marked difference in the symptoms was noticed; hence those observed on Crosby's Egyptian beets will be described as typical for all varieties.

In the early phases of symptom production, cleared veinlets (plate 5, *A*) developed in the same manner as that described on the young leaves of sugar-beet seedlings. Stunting, crinkling, small blisterlike elevations, and malformations on the youngest leaves (plate 5, *B*) sometimes occur, but as growth continued, normal-shaped leaves are produced and dwarfing is largely overcome. Interveneal chlorosis (plate 5, *H*) sometimes appears on the intermediate leaves, and a predominance of small, chlorotic dots and irregular areas (plate 5, *C*) margined with red were observed on the outer leaves.

The most striking symptom is rings margined with red, which begin to develop on the older leaves 10 days after inoculation. Small red rings each surrounding a chlorotic center (plate 5, *D*) may appear, which frequently fuse (plate 5, *E*). More often, however, large red rings, varying from less than 1 mm to 4 mm in diameter, occur on the outer leaves. Sometimes wide, outer red rings appear, each with an inner, chlorotic ring enclosing a pale-red center (plate 5, *F*). The rings frequently coalesce (plate 5, *G*). In a later stage of the disease, a necrotic center appears, which enlarges in the ring, and may drop out and leave a hole in the leaf.

The incubation period of the disease in the greenhouse varied from 6 days to 8 in the seven varieties of garden beets tested (table 1).

Swiss Chard. A departure from the cleared veinlets as the first symptom was noted in Large-ribbed White Swiss chard. The first symptom to appear on the youngest leaves is a few small, scattered, chlorotic dots (plate 7, *A*); these enlarge into chlorotic blotches involving most of the leaf surface. The older leaves usually show a predominance of chlorotic rings with green centers and finely striated borders (plate 6, *B*). Sometimes the chlorotic rings coalesce. Small chlorotic dots usually are intermingled with the rings.

A clearing of the veinlets (plate 7, *B*) was the first symptom to develop on Large-ribbed Dark Green and Lucullus varieties of Swiss chard, followed by interveneal chlorosis and mottling, and by chlorotic rings surrounding green centers (plate 6, *B*).

Naturally infected young Swiss chard obtained from San Pablo, September 30, 1936, showed chlorotic blotching and some rings, but was not noticeably stunted. Naturally infected old plants of Large-ribbed Swiss chard collected from Bay Farm Island on February 1, 1937, were severely stunted and malformed, with mottled leaves. After being potted and kept in the greenhouse, new leaves developed from the center and from adventitious shoots around the crown. Most of the young leaves were severely crinkled along the margins and malformed. Blisterlike elevations (plate 7, *C*), confined chiefly to the leaf margin, were common on the chlorotic leaves. Other leaves from the same plant were malformed (plate 7, *D*) but not mottled. The tips of the leaves may turn dark yellow or orange and necrosis occurs.

Three varieties of Swiss chard—Large-ribbed Dark Green, Large-ribbed White, and Lucullus—were inoculated with the juice from mosaic beets. The virus was recovered in 100 per cent of the trials, as shown in table 1. The inen-

bation period of the disease in the greenhouse varied from 6 to 19 days in the three varieties of Swiss chard infected with the sugar-beet-mosaic virus (table 1).

Spinach. Spinach was demonstrated to be naturally infected with beet mosaic in vegetable gardens near San Pablo. The virus extract from diseased spinach collected in the field was inoculated into healthy spinach and sugar-

beet plants, and typical symptoms of beet mosaic developed (plate 1, B). The symptoms are described in another paper (Severin, 1948).

The incubation of the disease varied from 7 to 15 days in the five varieties of spinach experimentally infected with beet mosaic (table 1).

Common Summer Cypress. The foliage symptoms of sugar-beet mosaic on common summer cypress were not evident, but the apical shoots of the branches were stunted within 9 days after inoculation. A slight upward curling of the tips of the long, narrow leaves occurs. Seven of 10 plants inoculated showed symptoms. Inoculations of the extract from the 10 plants experimentally infected to healthy sugar beets resulted in recovery of the virus in 5 of the 10 sugar beets inoculated, as shown in table 1.

New Zealand Spinach. Systemic infection of New Zealand spinach resulted when healthy plants were inoculated with juice from mosaic sugar beets. The first symptoms on young leaves are small, irregular, chlorotic flecks along and between the veins. Older leaves develop chlorotic areas and veinbanding along the midrib and lateral veins (plate 8, A). On some of the larger leaves the surface is stippled with small,



Fig. 1. Local symptoms of sugar-beet mosaic on the tobacco leaves on which the green peach aphid (*Myzus persicae*) had fed: A, Havana tobacco (*Nicotiana tabacum*) leaf showing chlorotic, circular areas, sometimes with a pinpoint necrotic center; B, Primus tobacco (*N. tabacum*) leaf showing concentric rings.

irregular, sunken dots (plate 8, B). In later stages of the disease, large, circular, chlorotic areas, 5 mm in diameter, occur on or between the veins of mature leaves. These rings become orange in color, each showing a darker, inner ring (plate 8, C), which later becomes necrotic. Sometimes necrosis of the circular areas spreads along the lateral veins to the leaf margin (plate 8, D). Growth of the apical and axillary shoots was retarded, but infected plants were still growing 8 weeks after inoculation.

Havana-Type and Primus Tobaccos. Hoggan (1933) failed to infect Havana-type tobacco by mechanical inoculation, but obtained local symptoms upon the leaves on which *Myzus persicae* and *Macrosiphum solanifolii* had fed; systemic infection did not follow. The virus was readily recovered from the tobacco leaves showing symptoms, by mechanically inoculating the extracted juice in the leaves of healthy sugar beets.

This work was repeated by feeding infective *Myzus persicae* on the leaves of a Havana-type variety and on Primus tobacco (*Nicotiana tabacum*). The leaves on which *M. persicae* was feeding showed circular, chlorotic areas, sometimes with a pinpoint, necrotic center (fig. 1, A), which is probably the feeding puncture. Leaves of Primus tobacco showed concentric rings (fig. 1, B). The virus extract from pieces of the leaves showing symptoms was inoculated into the leaves of healthy sugar-beet seedlings, and typical symptoms of sugar-beet mosaic developed.

PROPERTIES OF THE VIRUS

The results of studies on the properties of the sugar-beet-mosaic virus conducted by Hoggan (1933) and Pound (1947) in the United States and by Verplancke (1934-35) in Belgium are compared below:

	Hoggan	Verplancke	Pound
	+ -		
Longevity <i>in vitro</i>	24-48 hours (70° F)	6-7 days (20° C) 9-10 days (12° C)	72 hours (20° C)
Tolerance to dilution	1:1,000	1:100,000	1:2,000
	+ -	+ -	
Thermal death point	55°-60° C	90°-95° C	61° C (10 minutes)

The differences between the results of Hoggan and those of Verplancke were explained by the latter on the grounds that there was probably more than one virus concerned in the production of sugar-beet mosaic.

Quanjer (1936) considered the property studies of Hoggan (1933) to be reliable but those of Verplancke (1934-35) to be valueless.

Thermal Inactivation. Undiluted, extracted juice from the blades and petioles of experimentally infected sugar beets was used in determining the thermal inactivation of the virus. Extractions were made 1 to 7, and 179 days after the first appearance of symptoms of the disease. Ten cc of diseased juice was poured into thin-walled Pyrex test tubes, over the mouths of which four thicknesses of fish swim-bladder membrane were tightly drawn and made watertight by means of rubber bands. The tubes were submerged upright in a water bath controlled by an electric thermostat. The water was kept in circulation to maintain a uniform temperature with a motor-driven agitator. A thermometer with 0.5° C gradations was suspended in the water at the depth at which the tubes were held. Determinations were made at 5° C intervals. The time of exposure in the water bath was 11 minutes, allowing 1 minute for heat to penetrate the glass. After exposure to the desired temperature, the tubes were quickly cooled in running water and the extracts were then used for inoculation. Unheated controls were used in all tests. The number of infections obtained by mechanical inoculation into 5 healthy sugar-beet seedlings in each trial is shown in table 2.

As shown in table 2, the virus remained active after heating 10 minutes at 50° and 55°C but was inactivated after heating to 60°. No apparent difference was exhibited between trials when extractions were made 1 to 7, or 179 days from the time symptoms first appeared. The results agree with those reported by Hoggan (1933).

Effects of Freezing Virus Extract. Virus extracts were obtained from mosaic-infected sugar beets, 98 to 100 days after symptoms first appeared. Ten cc of expressed juice was placed in cotton-plugged test tubes and kept in

TABLE 2
THERMAL INACTIVATION OF SUGAR-BEET-MOSAIC VIRUS*

Temperature, °C	Beets inoculated	Beets infected	Per cent infected
Unheated control	25	23	92
50.	25	17	68
55.	25	7	28
60.	25	0	0
65.	25	0	0

* Combined results with extracts obtained 1 to 7 and 179 days after symptoms appeared.

TABLE 3
EFFECT OF FREEZING VIRUS EXTRACT OF SUGAR-BEET-MOSAIC KEPT IN COLD STORAGE AT -18° C

Age of virus extract, months	Number of beets		Per cent infected
	Inoculated	Infected	
Control.....	25	25	100
1.....	25	23	92
2.....	25	5	20
3.....	25	2	8
4.....	25	1	4
5.....	25	0	0

a darkened, cold-storage room at -18°C. Inoculations of diseased juice were made at the time of extraction to serve as controls for each trial. The results obtained are stated in table 3.

As shown in table 3, all control plants became infected, and a marked decrease in the number of infections occurred after exposure for 2 months to a freezing temperature. A single infection out of 25 beets inoculated was obtained after 4 months, and no infections occurred after 5 months in cold storage at -18°C.

Tolerance to Dilution. The tolerance to dilution of the virus was determined with the juice expressed from blades and petioles of experimentally infected sugar beets at intervals varying from 1 to 3 days to 118 days from the time that symptoms first appeared. The diluent consisted of sterile distilled water. Different pipettes were used with each dilution. An undiluted control was used in each trial. The results with extracts prepared 1 to 3 days after symptoms appeared are given in table 4.



Plate 1. Symptoms of sugar-beet mosaic: A, on *Beta maritima*, showing stunting of apical and axillary shoots, chlorotic dots on older leaves, necrosis and drying of youngest leaves, blackening of sepals and other flower parts, and necrotic streaks on stem; B, on Long Standing Bloomsdale spinach (*Spinacia oleracea*), showing small chlorotic areas and cleared veinlets.

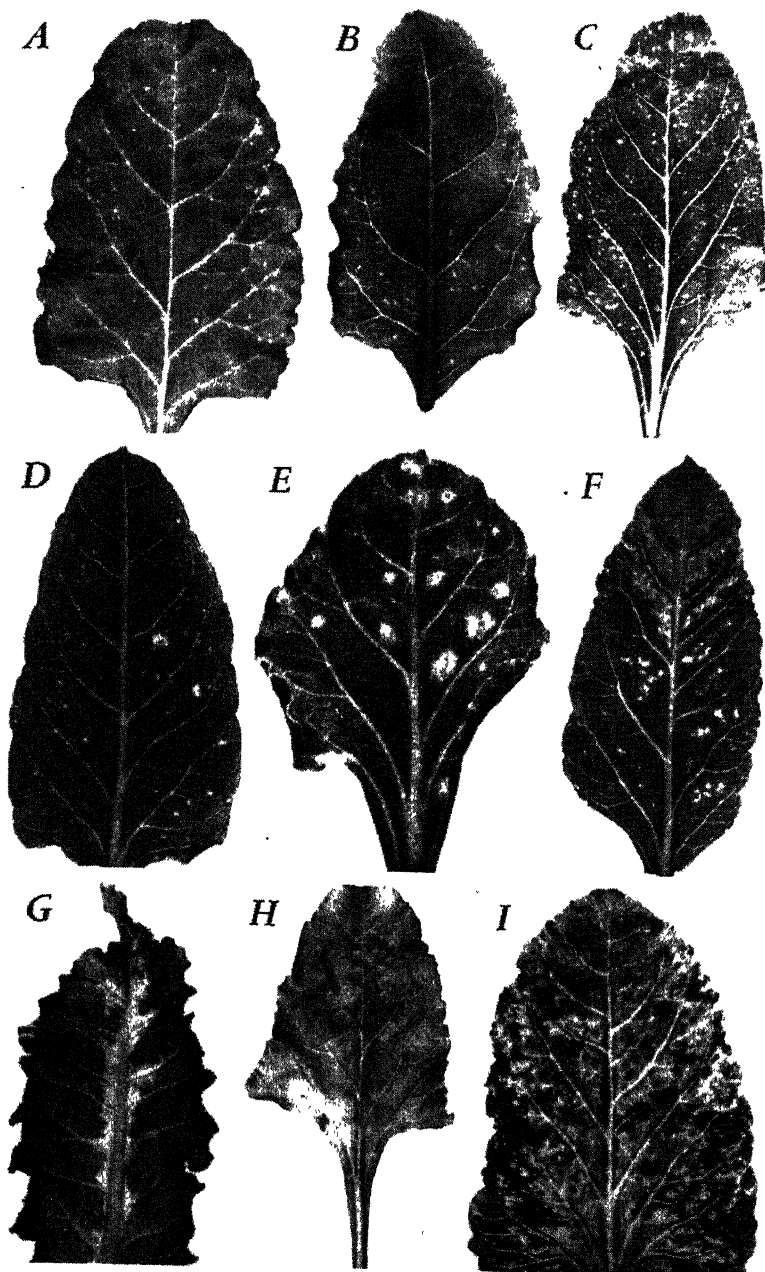


Plate 2. Sugar beet (*Beta vulgaris*) leaves showing symptoms of mosaic: *A*, minute yellow or pale-green flecks on youngest leaf, the first symptom of the disease; *B*, flecks and the veinlets beginning to clear; *C*, intermediate leaf, 3 weeks after inoculation, showing only chlorotic dots, taken from the same plant as the one that showed the ring pattern (plate 6, *A*); *D*, chlorotic dots and small, irregular, chlorotic areas; *E*, enlargements of chlorotic dots; *F*, interveinal chlorosis; *G*, chlorotic band extending along midrib and bases of lateral veins; *H*, necrosis of petiole and midrib; *I*, chlorosis of leaf, with chlorotic blotches.

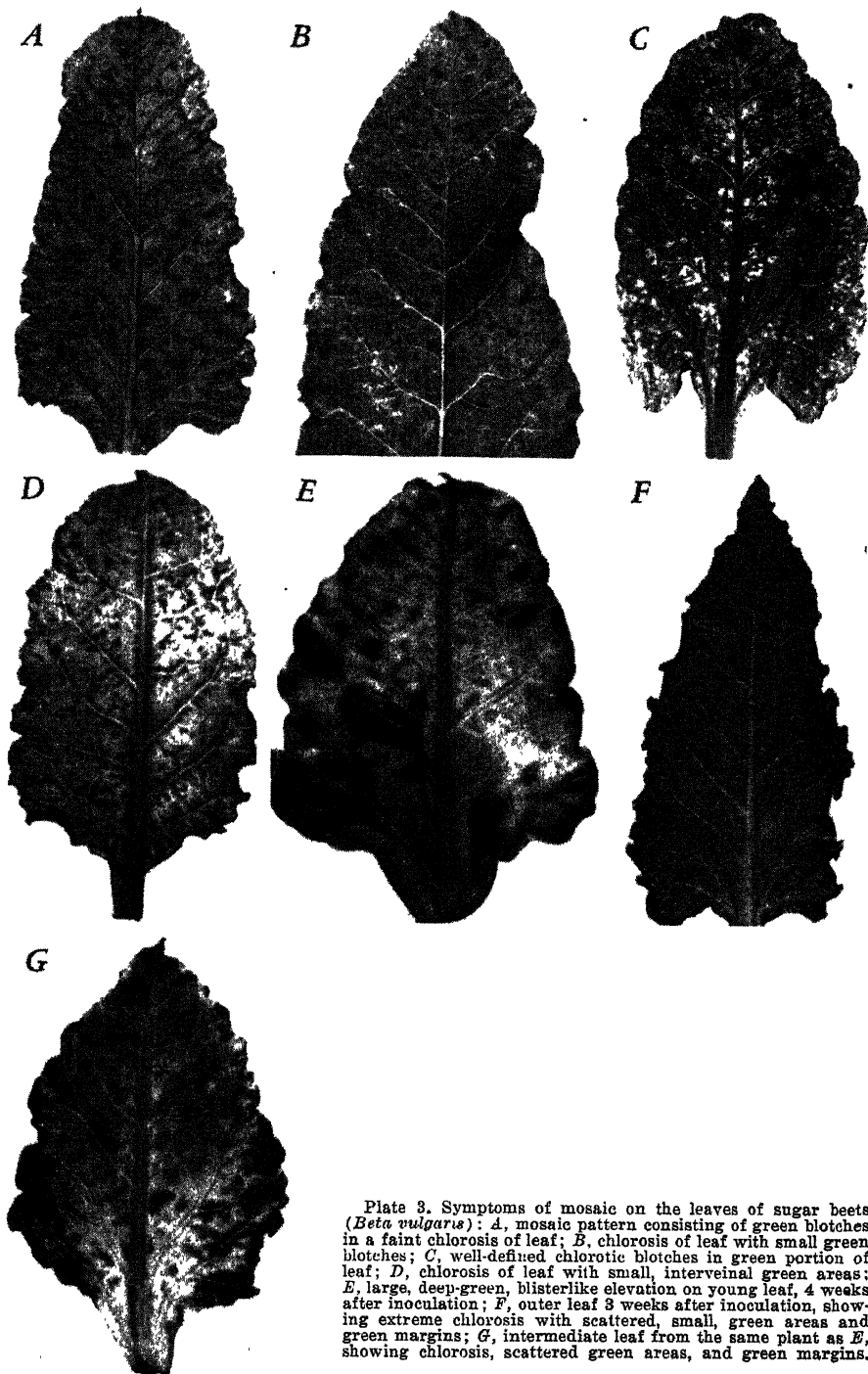


Plate 3. Symptoms of mosaic on the leaves of sugar beets (*Beta vulgaris*): *A*, mosaic pattern consisting of green blotches in a faint chlorosis of leaf; *B*, chlorosis of leaf with small green blotches; *C*, well-defined chlorotic blotches in green portion of leaf; *D*, chlorosis of leaf with small, interveinal green areas; *E*, large, deep-green, blisterlike elevation on young leaf, 4 weeks after inoculation; *F*, outer leaf 3 weeks after inoculation, showing extreme chlorosis with scattered, small, green areas and green margins; *G*, intermediate leaf from the same plant as *E*, showing chlorosis, scattered green areas, and green margins.

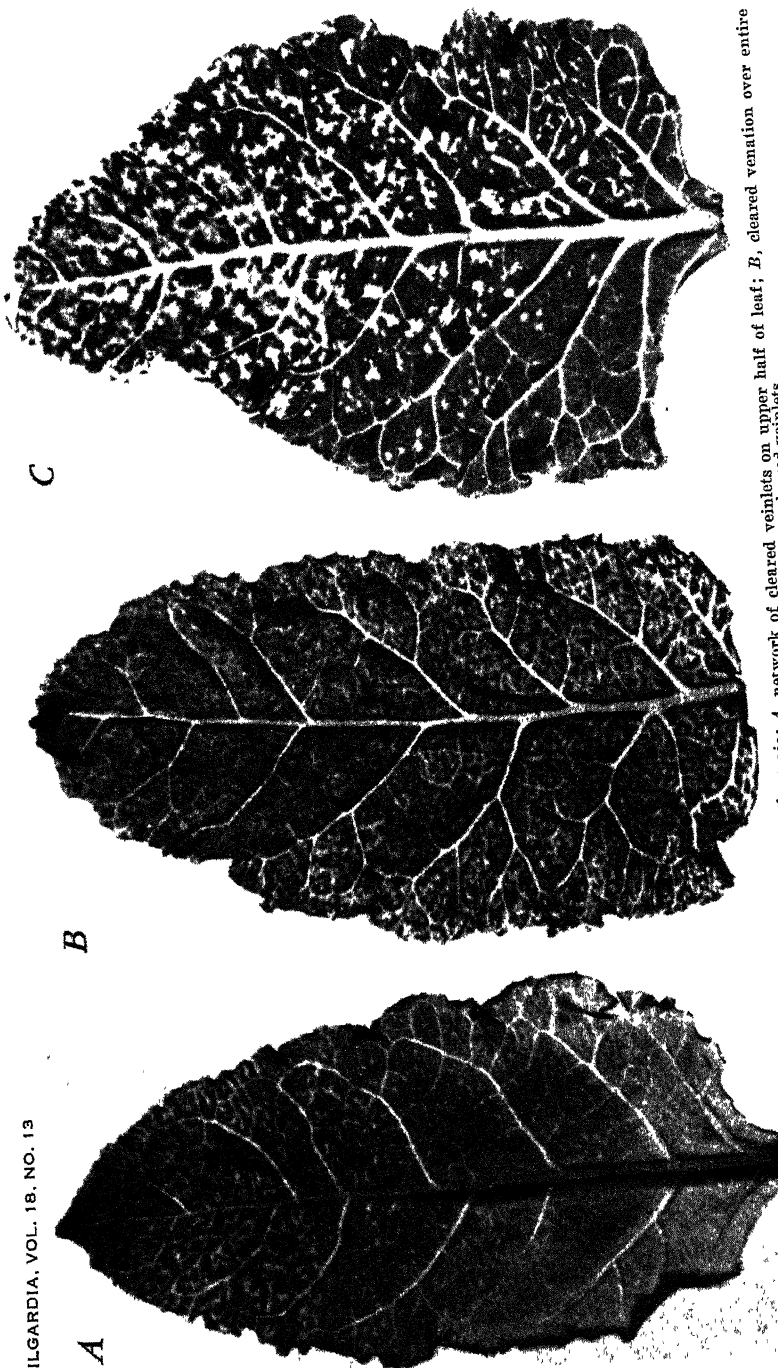


Plate 4. Sugar beet (*Beta vulgaris*) leaves showing symptoms of mosaic: A, network of cleared veins on upper half of leaf; B, cleared venation over entire leaf; C, widening and merging of chlorotic areas along cleared veins.

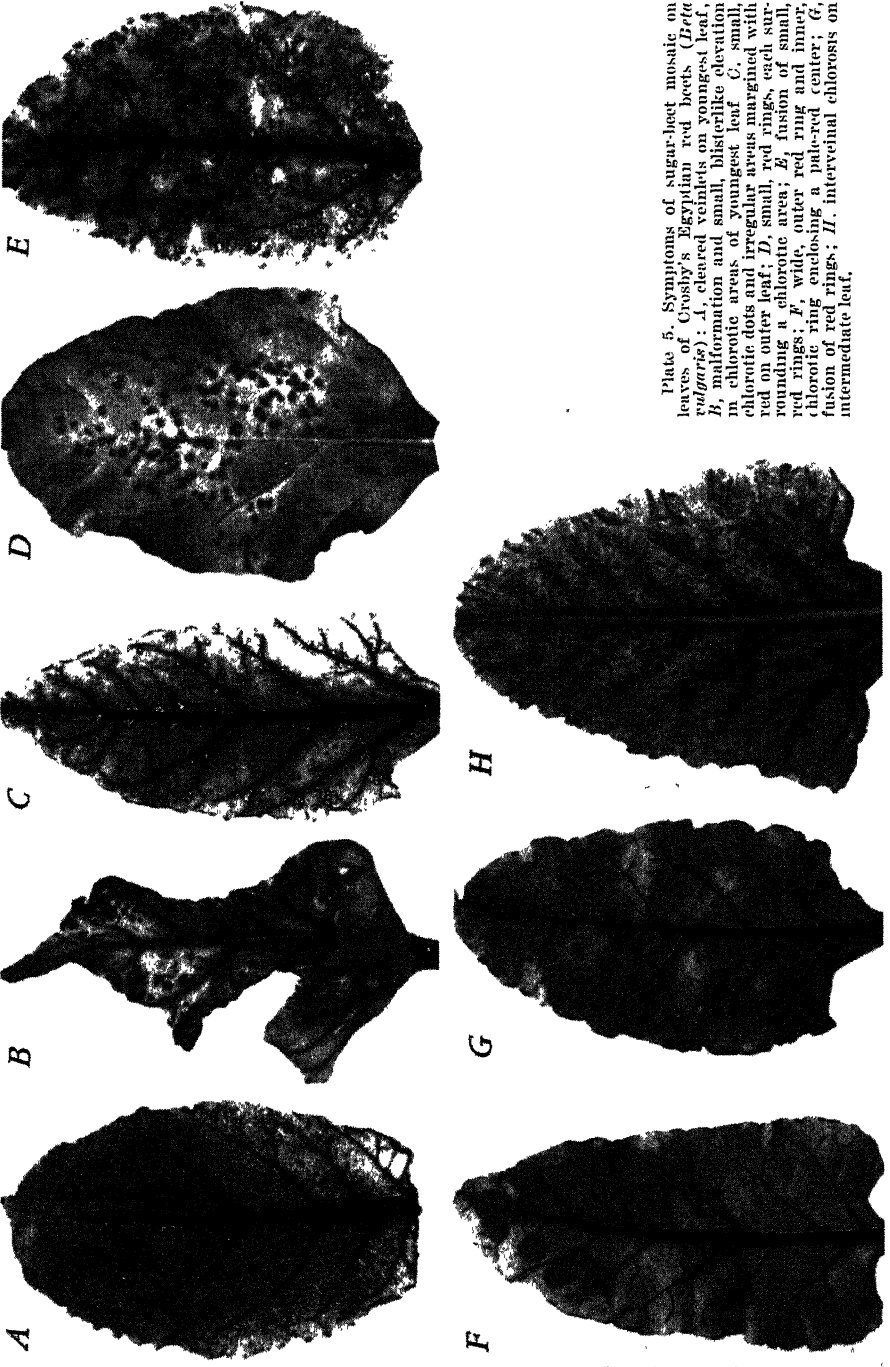


Plate 5. Symptoms of sugar-beet mosaic on leaves of Crosby's Egyptian red beets (*Beta vulgaris*): *A*, cleared venicles on youngest leaf, *B*, malformation and small, blisterlike elevation in chlorotic areas, *C*, small, chlorotic dots and irregular areas margined with red on outer leaf; *D*, small, red rings, each surrounding a chlorotic area; *E*, fusion of small, red rings; *F*, wide, outer red ring and inner, chlorotic ring enclosing a pale-red center; *G*, fusion of red rings; *H*, interveinal chlorosis on intermediate leaf.

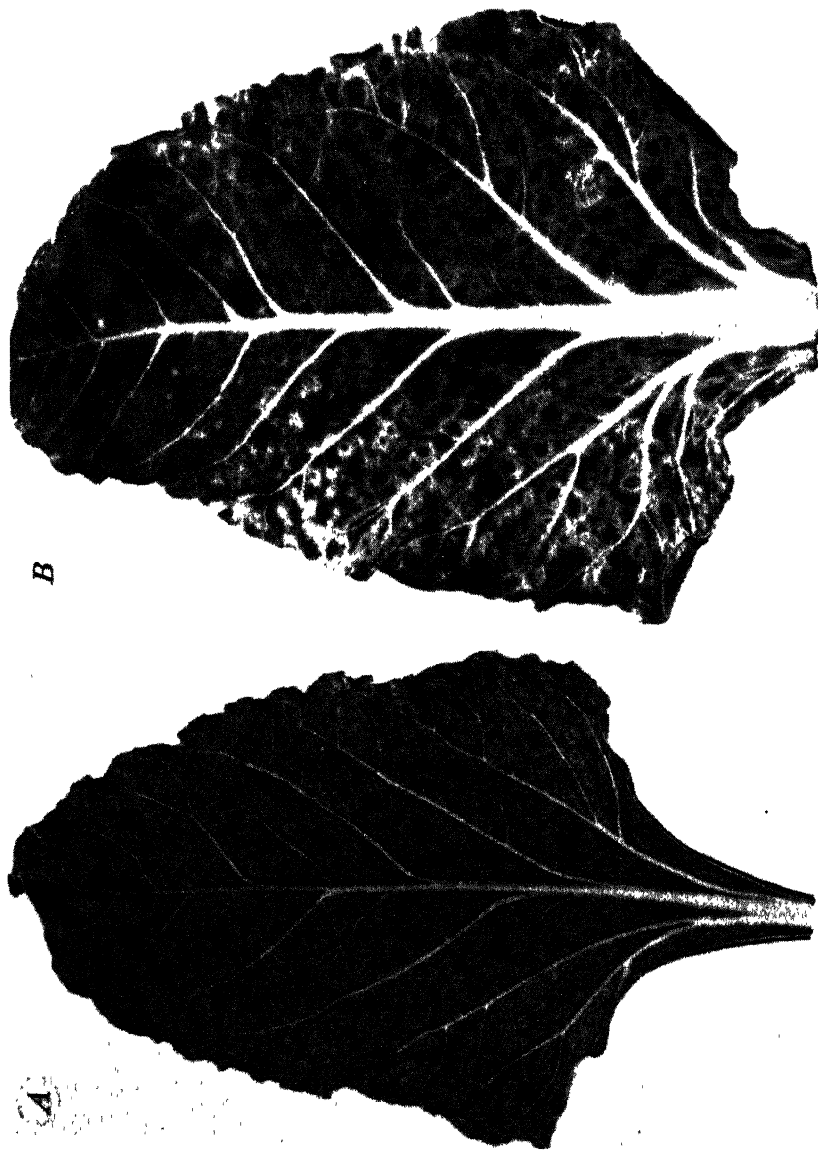


Plate 6. Symptoms of sugar-beet mosaic: *A*, on sugar beet (*Beta vulgaris*), showing chlorotic rings with green centers and numerous, scattered, chlorotic dots on intermediate leaf, 3 weeks after inoculation; *B*, on older leaf of Large-filled White Swiss chard (*Beta vulgaris* var. *cicla*), showing predominance of chlorotic rings with green centers and finely striated borders, some rings coalescing, and small chlorotic dots intermingled with the rings.

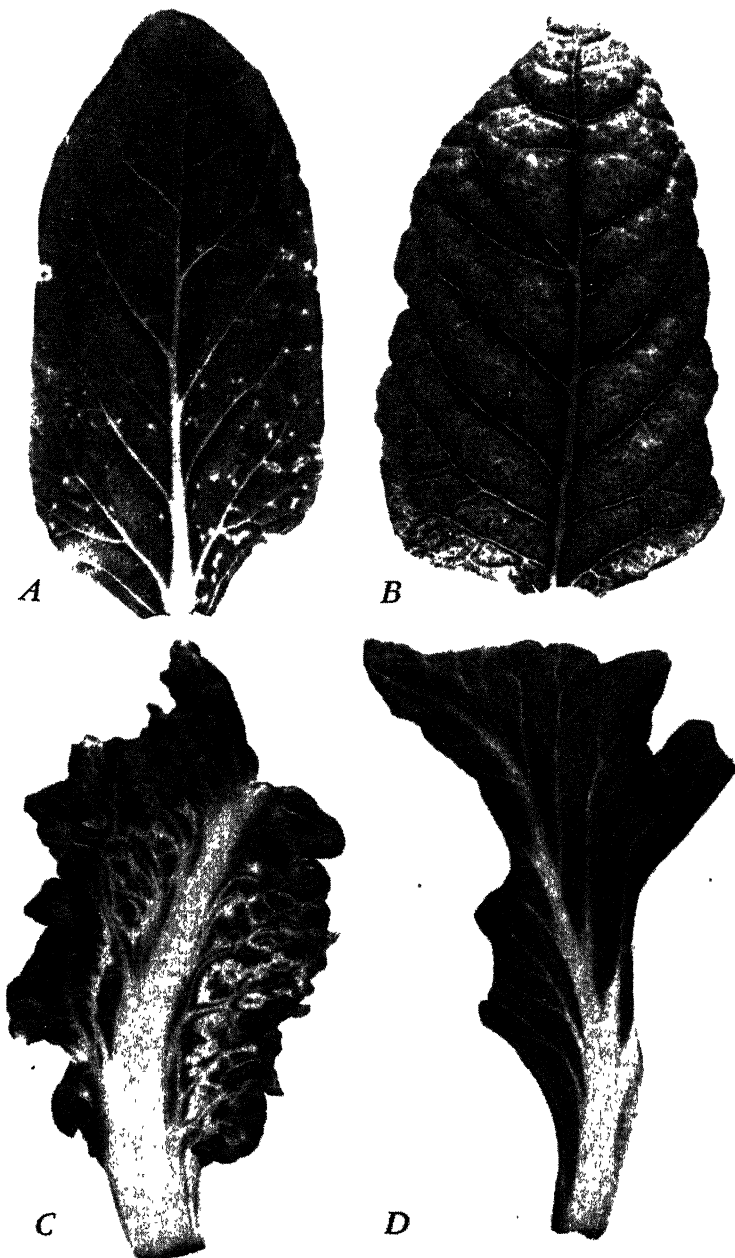


Plate 7. Varieties of Swiss chard (*Beta vulgaris* var. *cicla*) showing symptoms of sugar-beet mosaic: A, young leaf from the Large-ribbed White variety experimentally infected, showing scattered, small, chlorotic dots, the first symptom on this variety, 12 days after inoculation; B, young leaf from the Lucullus variety experimentally infected, showing cleared veinlets on entire leaf, the first symptom on this variety; C, young leaf from adventitious shoot of the Large-ribbed White variety naturally infected, showing malformation, crinkling of the margins, chlorosis, and blisterlike elevations; D, malformed leaf from another shoot showing no mosaic symptom.

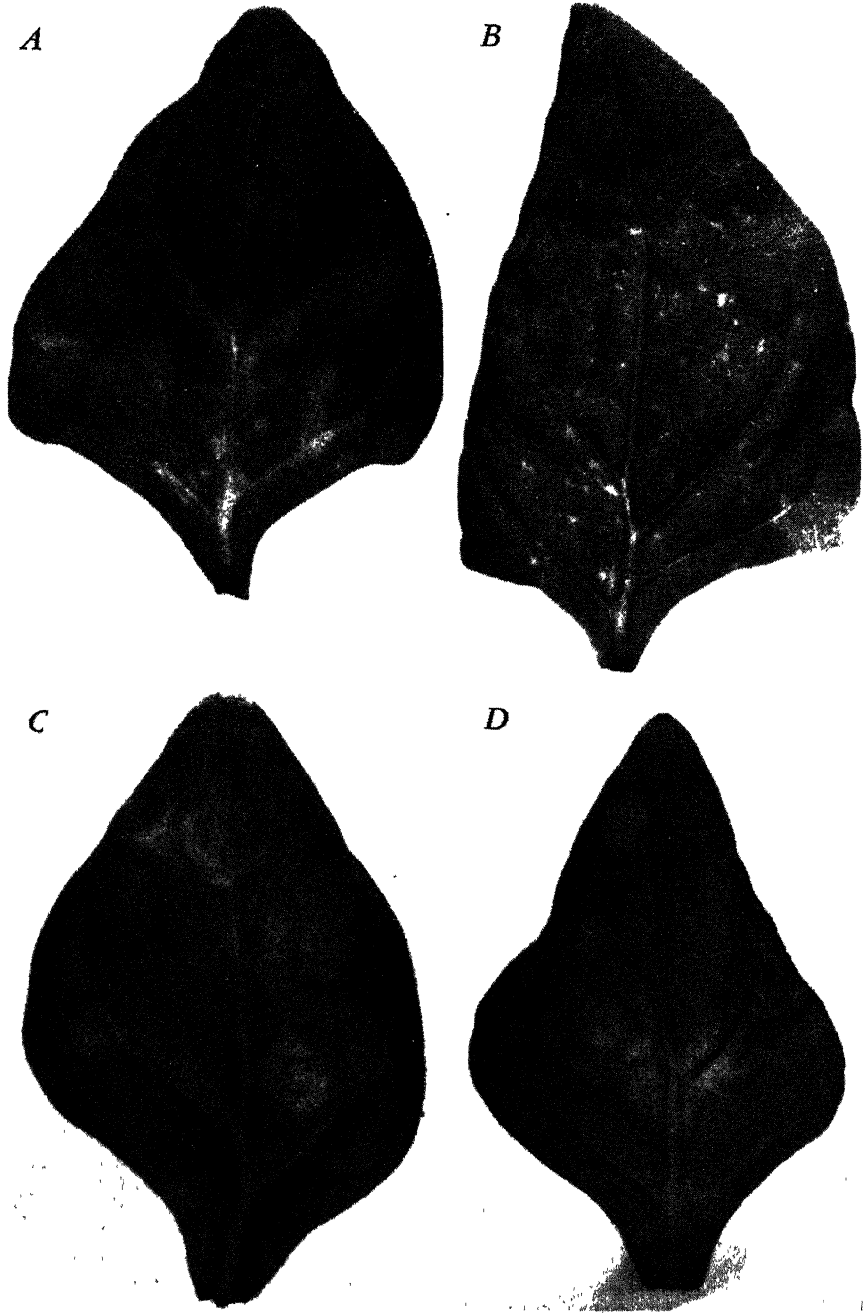


Plate 8.—Leaves of New Zealand spinach (*Tetragonia expansa*) showing symptoms of sugar-beet mosaic: A, older leaf showing chlorotic areas and veinbanding along the midrib and veins; B, outer leaf showing stippling with small, irregular, sunken dots; C, mature leaf showing large, circular, orange-colored areas, each with a darkened, inner ring; D, necrosis of circular area spreading along a lateral vein to the margin of the outer leaf.

As table 4 shows, tolerance to dilution of the virus extract was 1:5,000 for the extract obtained 1 to 3 days after symptoms first appeared. Extract obtained from plants 118 days after symptoms first developed gave no infections at a dilution of 1:500; and none was obtained at a dilution of 1:1,000 of extract from plants 107 days after symptoms were first evident. The results indicate that the dilution tolerance of the virus extract varied inversely with the number of days between the first appearance of symptoms and the extraction of the juice, though the same strain of virus was maintained throughout.

TABLE 4
TOLERANCE OF SUGAR-BEET-MOSAIC VIRUS* TO DILUTION

Dilution	Number of beets		Per cent infected
	Inoculated	Infected	
Undiluted control.....	145	125	86
1:10.....	30	22	73
1:100.....	65	33	51
1:500.....	40	36	90
1:1,000.....	95	36	38
1:2,000.....	65	14	22
1:3,000.....	65	5	8
1:4,000.....	65	4	6
1:5,000.....	120	4	3
1:6,000.....	25	0	0
1:7,000.....	25	0	0
1:8,000.....	25	0	0
1:9,000.....	25	0	0
1:10,000.....	145	1	1
1:15,000.....	85	1	1
1:20,000.....	85	1	1
1:25,000.....	50	0	0

* Extract obtained 1 to 3 days after symptoms first appeared

Differences between the results presented in table 4 and those of Hoggan (1933), who reported a dilution to tolerance of 1:1,000, may be due to the use of more recently infected beets in this experiment than were used in her studies.

In later experiments in which the virus extract was obtained from small sugar beets shortly after symptoms appeared, only 1 infection occurred at each of the following dilutions: 1:10,000, 1:15,000, and 1:20,000, and none at 1:25,000 (table 4). Since no infections were obtained with dilutions of 1:6,000, 1:7,000, 1:8,000, and 1:9,000, the results with greater dilutions must be considered as errors. The inoculated beets were placed in cages enclosed with small-mesh, brass screen wire, but these cages did not eliminate ants, which may have carried infective aphids.

Tolerance to Aging in Vitro. To determine the resistance of the virus to aging *in vitro*, test tubes containing 10 cc of expressed juice from the blades and petioles of experimentally infected sugar beets were plugged with cotton and kept in the dark at room temperature. Fresh virus extract was used as a control in each trial. Mechanical inoculations to healthy beet seedlings were made at intervals varying from 3 to 144 hours to determine the infectivity of the virus. The results are shown in table 5.

As appears in table 5, the virus was active after exposure for 96 hours *in vitro* at room temperature but lost its infectivity after aging 144 hours, or 6 days. No difference in results was found in virus extracts obtained from plants 1 to 7 days and 107 days after symptoms first appeared.

Hoggan (1933) reported that but 2 of 20 plants were infected after 24 hours' aging and that no infections were obtained after 48 hours.

INSECTS WHICH FAILED TO TRANSMIT VIRUS

All attempts to transmit the virus with insects exclusive of Aphididae failed. The following insects were tried: Say stinkbug, *Chlorochroa sayi* Stål, tarnished plant bug, *Lygus pratensis oblineatus* (Say); harlequin cabbage

TABLE 5
TOLERANCE OF SUGAR-BEET-MOSAIC VIRUS TO
AGING IN VITRO

Hours exposed	Number of beets		Per cent infected
	Inoculated	Infected	
0 (control).....	100	97	97
3.....	25	20	80
6.....	25	10	40
12.....	25	10	40
18.....	25	7	28
24.....	75	36	48
48.....	100	30	30
60.....	75	23	31
72.....	75	13	17
96.....	5	1	20
144.....	5	0	0

bug, *Murgantia histrionica* (Hahn); a white fly, *Asterochiton vittatus* (Quaintance); citrophilus mealybug, *Pseudococcus gahani* Green; short-winged aster leafhopper, *Macrostelus divisus* (Uhler); western potato leafhopper, *Empoasca abrupta* DeLong; and a cercopid, *Cixius cultus* Ball.

APHID TRANSMISSION OF THE VIRUS

Vectors Reared on Mosaic-Infected Sugar Beets. A comparison was made of the transmission of the virus by three aphid species reared on experimentally infected beets and by mechanical inoculation. The species used in this test were erigeron root aphid, *Aphis middletonii* (Thomas); pea aphid, *Macrosiphum pisi* (Kaltenbach); and green peach aphid, *Myzus persicae* (Sulzer). Each species was colonized on 5 infected sugar beets. The aphids were transferred in lots of 20 from each infected beet to 5 healthy beets, making a total of 25 trials for each species. In order to compare aphid transmission with mechanical inoculation, the virus extract from the infected plants on which the aphids had been reared was inoculated into 5 healthy beets.

As table 6 shows, *Aphis middletonii*, *Macrosiphum pisi*, and *Myzus persicae* transmitted the virus in 20, 60, and 56 per cent of the trials respectively. The infections obtained by mechanical inoculation averaged 98 per cent in all cases.

In another experiment, bean or dock aphid, *Aphis rumicis* Linnaeus, was colonized on 15 mosaic beets. As in the preceding experiment, the aphids were transferred in lots of 20 from each infected beet to 5 healthy beets; there was thus a total of 75 tests. Not a single infection resulted. Then 100 bean aphids

TABLE 6

TRANSMISSION OF SUGAR-BEET-MOSAIC VIRUS BY APHIDS REARED ON
INFECTED BEETS AND BY MECHANICAL INOCULATION

Aphid transmission			Mechanical inoculation†	
Aphid species and lot* no.	Beets inoculated	Beets infected	Beets inoculated	Beets infected
<i>Aphis middletonii</i> :				
Lot 1.	5	3	5	5
Lot 2.	5	1	5	5
Lot 3.	5	1	5	5
Lot 4.	5	0	5	5
Lot 5.	5	0	5	4
Total....	25	5	25	24
Percentage....	..	20	..	96
<i>Macrosiphum pisi</i> :				
Lot 1.	5	4	5	5
Lot 2.	5	4	5	5
Lot 3.	5	3	5	5
Lot 4.	5	2	5	5
Lot 5.	5	2	5	4
Total....	25	15	25	24
Percentage..	..	60	..	96
<i>Myzus persicae</i> :				
Lot 1.	5	5	5	5
Lot 2.	5	4	5	5
Lot 3.	5	4	5	5
Lot 4.	5	3	5	5
Lot 5.	5	3	5	5
Lot 6.	5	3	5	5
Lot 7.	5	2	5	4
Lot 8.	5	2	5	4
Lot 9.	5	1	5	5
Lot 10.	5	1	5	5
Total.....	50	28	50	48
Percentage....	..	56	..	96

* 20 aphids per lot.

† Virus extract for mechanical inoculation was taken from the same diseased beet on which the corresponding lot of aphids fed.

reared on mosaic beets were transferred singly to healthy beets. One infection was obtained. It is evident that the bean aphid rarely transmits the virus.

Vectors Reared on Other Host Plants. Eleven species of aphids which have not been found to multiply on beets under natural conditions were tested, and found to be capable of transmitting the virus. The transmission of the virus from experimentally infected to healthy beets by ten of these species,

TABLE 7
TRANSMISSION OF SUGAR-BEET-MOSAIC VIRUS TO BEETS BY APHIDS
REARED ON OTHER HOSTS AND BY MECHANICAL INOCULATION

Aphid transmission				Mechanical inoculation†	
Aphid species, plant it was reared on, and lot* no.	Period on diseased beet, days	Beets inoculated	Beets infected	Beets inoculated	Beets infected
<i>Aphis api-graveolens</i> reared on celery:					
Lot 1.....	2	5	5	5	5
Lot 2.....	2	5	4	5	5
Lot 3.....	2	5	4	5	5
Lot 4.....	2	5	3	5	5
Lot 5.....	2	5	3	5	5
Total.....	..	25	19	25	25
Percentage.....	..	.	76	..	100
<i>Aphis apii</i> reared on celery:					
Lot 1.....	2	5	4	5	4
Lot 2.....	2	5	4	5	5
Lot 3.....	2	5	3	5	5
Lot 4.....	2	5	3	5	5
Lot 5.....	2	5	2	5	5
Total.....	..	25	16	25	24
Percentage.....	64	..	96
<i>Aphis ferruginea-striata</i> reared on celery:					
Lot 1.....	2	5	2	5	5
Lot 2.....	2	5	0	5	5
Lot 3.....	2	5	0	5	5
Lot 4.....	2	5	0	5	5
Lot 5.....	2	5	0	5	5
Total.....	..	25	2	25	25
Percentage.....	8	..	100
<i>Aphis gossypii</i> reared on celery:					
Lot 1.....	2	5	4	5	5
Lot 2.....	2	5	4	5	5
Lot 3.....	2	5	2	5	5
Lot 4.....	2	5	2	5	5
Lot 5.....	2	5	2	5	5
Total.....	..	25	14	25	25
Percentage.....	56	..	100
<i>Aphis medicaginis</i> reared on California privet:					
Lot 1.....	2	5	4	5	5
Lot 2.....	2	5	2	5	4
Lot 3.....	2	5	2	5	5
Lot 4.....	2	5	2	5	4
Lot 5.....	2	5	1	5	4
Total.....	..	25	11	25	22
Percentage.....	44	..	88

* 20 aphids per lot.

† Virus extract for mechanical inoculation was taken from the same diseased beet on which the corresponding lot of aphids fed.

TABLE 7—Continued

Aphid transmission				Mechanical inoculation†	
Aphid species, plant it was reared on, and lot* no.	Period on diseased beet, days	Beets inoculated	Beets infected	Beets inoculated	Beets infected
<i>Aphis pomi</i> reared on <i>Rumex crispus</i> :					
Lot 1.....	1	5	3	5	4
Lot 2.....	1	5	1	5	5
Lot 3.....	1	5	1	5	5
Lot 4.....	1	5	0	5	5
Lot 5.....	1	5	0	5	5
Total.....	..	25	5	25	24
Percentage.....	20	..	96
<i>Cavariella capreae</i> reared on celery:					
Lot 1.....	2	5	1	5	4
Lot 2.....	2	5	1	5	5
Lot 3.....	2	5	0	5	5
Lot 4.....	2	5	0	5	5
Lot 5.....	2	5	0	5	4
Total.....	..	25	2	25	23
Percentage.....	8	..	92
<i>Myzus solani</i> reared on celery:					
Lot 1.....	2	5	4	5	5
Lot 2.....	2	5	2	5	5
Lot 3.....	2	5	2	5	5
Lot 4.....	2	5	2	5	5
Lot 5.....	2	5	1	5	4
Total.....	..	25	11	25	24
Percentage.....	44	..	96
<i>Rhopalosiphum conii</i> reared on <i>Conium maculatum</i> :					
Lot 1.....	1	5	2	5	4
Lot 2.....	1	5	1	5	5
Lot 3.....	1	5	1	5	5
Lot 4.....	1	5	1	5	5
Lot 5.....	1	5	0	5	5
Total.....	..	25	5	25	24
Percentage.....	20	..	96
<i>Rhopalosiphum pseudobrassicae</i> reared on stock:					
Lot 1.....	1	5	2	5	5
Lot 2.....	1	5	1	5	5
Lot 3.....	1	5	0	5	5
Lot 4.....	1	5	0	5	5
Lot 5.....	1	5	0	5	5
Total.....	..	25	3	25	25
Percentage.....	12	..	100

* 20 aphids per lot.

† Virus extract for mechanical inoculation was taken from the same diseased beet on which the corresponding lot of aphids fed.

reared on other host plants, was compared with mechanical inoculations. Previously noninfective aphids were fed on 5 infected beets from 24 to 48 hours, then were transferred in lots of 20 to each of 5 healthy beets. The virus extract from each infected plant on which the aphids had fed was inoculated into 5 healthy beets. The aphids that transmitted the virus were:

Celery leaf aphid, *Aphis apigraveolens* Essig
 Celery aphid, *Aphis apii* Theobald⁷
 Rusty-banded aphid, *Aphis ferruginea-striata* Essig
 Cotton or melon aphid, *Aphis gossypii* Glover
 Bur clover or cowpea aphid, *Aphis medicaginis* Koch
 Green apple aphid, *Aphis pomi* De Geer
 Cabbage aphid, *Brevicoryne brassicae* (Linnaeus)
 Yellow willow aphid, *Cavariella capreae* (Fabricius)
 Turnip or false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis)
 Foxglove aphid, *Myzus solani* (Kaltenbach)
 Honeysuckle aphid, *Rhopalosiphum conii* (Davidson)

As shown in table 7, *Aphis apigraveolens* fed readily on beets and transmitted the virus to 76 per cent of the plants, a higher percentage than that obtained with any of the three species reared on infected beets reported in table 6. The transmission of the virus by *Aphis apii*, *A. gossypii*, *A. medicaginis*, and *Myzus solani* was 64, 56, 44, and 44 per cent respectively (table 7). They also fed readily on beets even though reared on other plants.

Essig (1934) stated that *Aphis gossypii* attacks a wide variety of plants, including spinach; and Gillette and Palmer (1931-1934) reported that *A. medicaginis* occurs on *Kochia* in the family Chenopodiaceae. It is not improbable that the species of aphids which have been reported on plants of the Chenopodiaceae other than beets, as well as those species which were observed to feed readily on beets, may play a significant part in spread of the virus in beet fields, or from beets to weeds, and vice versa.

Transmission of the virus by the *Aphis ferruginea-striata*, *A. pomi*, *Cavariella capreae*, *Rhopalosiphum conii*, and *Rhopalosiphum pseudobrassicae* was 8, 20, 8, 20, and 25 per cent respectively (table 7). It was noted, however, that these species did not readily feed on beets and were not inclined to stay long on them. It is possible, therefore, that more infections did not result because few or no aphids fed on the infected or on the healthy beets.

Five trials from each of 20 infected beets, or a total of 100 tests, were made with lots of 20 cabbage aphids, *Brevicoryne brassicae*; 7 beets developed symptoms of the disease.

Twenty-five lots of 20 beet aphids, *Prociphilus betae* (Doane), transferred from the roots of mosaic to healthy beets, failed to transmit the virus.

A summary of the percentages of infections obtained with aphid species reared on mosaic beets and other host plants is shown in table 8.

MASS INOCULATION

In the opinion of nearly all entomologists and plant pathologists, a single aphid or leafhopper of a lot may cause infection; in other words, one of a group may inject the infective dose of a virus into a plant, and the other insects may play no role in producing the disease.

⁷ According to E. O. Essig (personal interview), *Aphis apii* Theobald may be identical with *A. helianthi* Monell.

TABLE 8
SUMMARY OF TRANSMISSION OF SUGAR-BEET-MOSAIC VIRUS BY
APHID SPECIES WITH LOTS OF 20 APHIDS IN EACH TEST

Common and scientific name	Number of beets		Per cent infected
	Inoculated	Infected	
Aphids reared on diseased beets:			
Erigeron root aphid (<i>Aphis middletonii</i>)	25	5	20
Pea aphid (<i>Macrosiphum pisi</i>)	25	15	60
Green peach aphid (<i>Myzus persicae</i>)	50	28	58
Aphids reared on other host plants:			
Cabbage aphid (<i>Brevicoryne brassicae</i>)	100	7	7
Celery leaf aphid (<i>Aphis apigraveolens</i>)	25	19	76
Celery aphid (<i>Aphis apii</i>)	25	16	64
Rusty-banded aphid (<i>Aphis ferruginea-striata</i>)	25	2	8
Cotton or melon aphid (<i>Aphis gossypii</i>)	25	14	56
Bur clover or cowpea aphid (<i>Aphis medicaginis</i>)	25	11	44
Green apple aphid (<i>Aphis pomi</i>)	25	5	20
Yellow willow aphid (<i>Cavariella capreae</i>)	25	2	8
Foxglove aphid (<i>Myzus solani</i>)	25	11	44
Honeysuckle aphid (<i>Rhopalosiphum conti</i>)	25	5	20
Turnip or false cabbage aphid <i>Rhopalosiphum pseudobrassicae</i>)	25	3	12

TABLE 9
TRANSMISSION OF SUGAR-BEET-MOSAIC VIRUS BY LOTS OF
VARYING NUMBERS OF *MYZUS PERSICAE* TRANSFERRED
FROM MOSAIC TO HEALTHY BEETS

Number of aphids in each lot	Number of lots on each diseased beet	Number of beets		Per cent infected
		Inoculated	Infected	
Test 1: 10 lots of each number from each of 5 diseased beets				
1 aphid.....	10	50	0	0
5 aphids.....	10	50	3	6
10 aphids.....	10	50	10	20
20 aphids.....	10	50	18	36
40 aphids.....	10	50	37	74
Test 2: 5 lots of each number from each of 10 diseased beets				
1 aphid.....	5	50	2	4
5 aphids.....	5	50	2	4
10 aphids.....	5	50	4	8
20 aphids.....	5	50	10	20
40 aphids.....	5	50	18	36

By Varying Numbers of Aphids. The green peach aphid, *Myzus persicae*, was chosen in the following experiments because of its readiness in colonizing on sugar beets and facility in handling. Populations of *M. persicae* were reared on 5 experimentally infected beets, from each of these, 10 lots each of 1, 5, 10, 20, and 40 apterous aphids were transferred to healthy beets. The results appear in table 9.

As shown in this table, there was a definite tendency toward an increase in number of infections obtained when the number of aphids was increased. No infections were obtained with 50 single aphids, while 6 per cent resulted with 5 aphids, 20 per cent with 10 aphids, 36 per cent with 20 aphids, and 74 per cent with 40 aphids in each lot.

This experiment was repeated with *Myzus persicae* reared on 10 experimentally infected sugar beets, from each of which 5 lots each of 1, 5, 10, 20,

TABLE 10
SHORT PERIODS OF TRANSMISSION OF SUGAR-BEET-MOSAIC
VIRUS BY VARYING NUMBERS OF *MYZUS PERSICAE*

Number of aphids on each beet	Minutes on infected beet	Minutes on healthy beet	Number of beets		Per cent infected
			Inoculated	Infected	
3 days after symptoms developed					
1.....	5	5	10	0	0
2.....	5	5	10	1	10
3.....	5	5	10	3	30
4.....	5	5	10	3	30
5.....	5	5	10	5	50
15 days after symptoms developed					
1.....	5	5	10	0	0
2.....	5	5	10	4	40
3.....	5	5	10	2	20
4.....	5	5	10	5	50
5.....	5	5	10	4	40
Results summarized					
1.....	5	5	20	0	0
2.....	5	5	20	5	25
3.....	5	5	20	5	25
4.....	5	5	20	8	40
5.....	5	5	20	9	45

and 40 aphids were transferred to healthy beets. These results also appear in table 9. The percentage of infection increased from 4 per cent with 1 or 5 aphids per plant to 36 per cent with 40 aphids. These results, however, do not prove mass inoculation.

By Single Aphids in Short Feeding Time. An experiment was conducted on virus transmission by *Myzus persicae* in short feeding periods on mosaic and healthy beets. Lots of 1, 2, 3, 4, and 5 previously noninfective, mature, apterous aphids were fed for 5 minutes on an infected beet 3 days after symptoms developed, and then on healthy beets for the same length of time. The same procedure was repeated again, 15 days after the first symptom appeared on the same beet.

Table 10 shows that no infections resulted when single aphids were used, and in general, the percentages of infections increased with the number of aphids used. No significant differences were noted between the results obtained

3 days and 15 days after symptoms had developed on the original infected beet. An average of 25 per cent infection was obtained with lots of 2 and 3 aphids, 40 per cent with 4, and 45 per cent with 5 aphids, as shown in the summarized results in table 10. Again there was no evidence to prove mass inoculation in this experiment.

RETENTION OF VIRUS

An experiment was conducted to determine how long *Myzus persicae* would retain the virus. Lots of 20 previously noninfective aphids were kept 1 hour on 10 different mosaic beets, then each lot was transferred hourly to 8 successive healthy beets, and maintained 15 hours on the ninth healthy beet.

TABLE 11
RETENTION OF SUGAR-BEET-MOSAIC VIRUS BY LOTS OF *MYZUS*
PERSICAE TRANSFERRED HOURLY TO SUCCESSIVE
HEALTHY SUGAR BEETS

Lot no.	Number of aphids on first plant	Results* on successive plants, with hourly transfers								Last infection produced by aphids, hour
		1st	2d	3d	4th	5th	6th	7th	8th	
1	20.....	+	+	-	-	-	-	-	-	3d
2	20.....	+	-	-	-	-	-	-	-	2d
3	20.....	+	-	-	-	-	-	-	-	2d
4	20.....	+	-	-	-	-	-	-	-	2d
5	20.....	+	-	-	-	-	-	-	-	2d
6	20.....	+	-	-	-	-	-	-	-	2d
7	20.....	-	+	-	-	-	-	-	-	3d
8	20.....	-	+	-	-	-	-	-	-	3d
9	20.....	-	+	-	-	-	-	-	-	3d
10	20.....	-	-	+	-	-	-	-	-	4th
	Total +.....	6	4	1	0	0	0	0	0	..
	Total -.....	4	6	9	10	10	10	10	10	..

* The plus sign (+) indicates the production of the disease, and the minus sign (-) shows that no disease resulted. Aphids were left 15 hours on the eighth plant.

As table 11 shows, infections occurred in 6 of 10 tests within the first hour, 3 within the second hour, and 1 within the third hour. One lot infected two successive plants during the first and second hours. No transmission of the virus was obtained after the third hour. It is possible, however, that with a lowering of the temperature during the winter, the aphids may retain the infectivity longer than 3 hours under natural conditions.

LOSS AND RECOVERY OF INFECTIVITY BY APHIDS ON INOCULATED PLANTS

An attempt was made to determine whether *Myzus persicae* was able to recover the virus from infected sugar beets before the first symptom of the disease developed. Large numbers of aphids were transferred from populations reared on 5 experimentally infected beets to 5 large healthy beets for a period of 2 days. From the third to the fourteenth day, lots of 20 of these aphids were transferred from each plant so inoculated to healthy beets.

The loss and recovery of infectivity by aphids on beets which they inocu-

lated with the virus, and the incubation period of the disease, or the period for the first symptom to develop, is shown in table 12. The elapsed time to the first recovery of the virus by aphids from the original infected beet varied from 8 to 12 days. The incubation period of the disease in the original infected beets varied from 8 to 12 days. A comparison of the first recovery of the virus by lots of 20 aphids with the incubation period of the disease in the original infected plants shows that only 1 lot of aphids recovered the virus before symptoms of the disease developed (1 day before), 1 lot recovered the virus on the same day that the first symptom appeared, and 3 lots recovered the virus in from 1 to 2 days after the earliest symptom developed.

TABLE 12
LOSS AND RECOVERY OF INFECTIVITY BY *MYZUS PERSICAE* ON SUGAR BEETS WHICH THEY INOCULATED WITH SUGAR-BEET-MOSAIC VIRUS

Original plant number	Results* on successive healthy beets, with aphids left 1 day on each beet (20 aphids per lot)												Days to the first symptom on original plant
	No. 1 (3d day)	No. 2 (4th day)	No. 3 (5th day)	No. 4 (6th day)	No. 5 (7th day)	No. 6 (8th day)	No. 7 (9th day)	No. 8 (10th day)	No. 9 (11th day)	No. 10 (12th day)	No. 11 (13th day)	No. 12 (14th day)	
1.....	-	-	-	-	-	+	-	+	+	+	-	+	9
2.....	-	-	-	-	-	-	-	+	-	+	-	+	8
3.....	-	-	-	-	-	-	-	+	+	-	+	+	9
4.....	-	-	-	-	-	-	-	+	-	-	+	-	8
5.....	-	-	-	-	-	-	-	-	-	+	+	-	12
Total +.....	0	0	0	0	0	1	1	4	2	3	3	3	
Total -.....	5	5	5	5	5	4	4	1	3	2	2	2	

* The plus sign (+) indicates the production of the disease, and the minus sign (-) shows that no disease resulted.

ATTEMPT TO TRANSMIT VIRUS WITH CORNICLE EXUDATE

No Malpighian tubules are known to occur in any species of aphids, and some of the waste products are probably eliminated in the cornicle exudate. Extensive tests were made to determine whether the cornicle exudate from infective *Myzus persicae*, possibly containing the virus, would cause the disease. Infective aphids were reared on a mosaic beet; each was touched gently on the abdomen with the point of a needle, and the cornicle exudate excreted was inoculated into the petioles, midrib, or veins of healthy beet seedlings. Five leaves of each beet were inoculated, each with one droplet from a different aphid. Fifty beet seedlings inoculated remained healthy.

SEPARATION OF MULTIPLE VIRUSES

Sugar beets showing symptoms of both mosaic and curly top on the same plant are common in the field, especially when outbreaks of aphids and the beet leafhopper, *Eutettix tenellus* (Baker), occur. When previously noninfective *Myzus persicae* and the beet leafhopper were exposed to a sugar beet containing the two viruses, *M. persicae* transmitted only the mosaic virus and the beet leafhopper only the curly-top virus to healthy sugar beets, as reported

in a previous paper (Severin, 1929). The two viruses thus were separated from a virus complex.

When the expressed juice from the leaves of a sugar beet containing the two viruses is inoculated into healthy sugar beets, an infection of only beet mosaic occurs by mechanical inoculation with the carborundum method. However, in an experiment previously reported (Severin, 1924), curly-top infection occurred with 9 out of 100 healthy beets when the virus extract from curly-top beets was inoculated into the crown with a flamed needle.

DESCRIPTION OF VIRUS

Name: Sugar-beet mosaic.

Host families: Chenopodiaceae, Aizoaceae, and Solanaceae.

Symptoms of disease: minute yellow or pale-green flecks, followed by vein clearing on youngest leaves, mottling followed by chlorosis, chlorotic rings with green centers on intermediate leaves, rarely deep-green blisterlike elevations, stunting of young plants, sometimes necrosis of petioles, midrib, and lateral veins under natural conditions.

Incubation period of disease: average 25.2 days outdoors during the autumn.

Properties of virus: thermal inactivation 60°C in 10-minute exposures; tolerance to dilution 1 : 5,000; resistance to aging *in vitro* 6 days.

Modes of transmission: Mechanical inoculation with expressed juice, 4 aphid species reared on sugar beets, 11 aphid species reared on other host plants.

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APPENDIX TO CITATIONS

Brief notes of the occurrences of sugar-beet mosaic in the United States have appeared in the Plant Disease Reporter.⁸ Frequently the collaborators of these reports were not mentioned, and it was found more convenient to list them in the chronological order rather than under the name of the collaborators and editors.

- 1921a. The Plant Disease Reporter 5(9):139.
1921b. The Plant Disease Reporter Suppl. 16:265-66.
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1926. The Plant Disease Reporter Suppl. 45:141.
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1928a. The Plant Disease Reporter Suppl. 59:85.
1928b. The Plant Disease Reporter Suppl. 61:294.
1929. The Plant Disease Reporter Suppl. 68:108.
1930a. The Plant Disease Reporter 14(17):176.
1930b. The Plant Disease Reporter Suppl. 75:55-56.
1936. The Plant Disease Reporter 20(15):231.
1943. The Plant Disease Reporter 28(36):643.
1944. The Plant Disease Reporter 28(36):1095-96.

⁸ A mimeographed pamphlet issued by the United States Bureau of Plant Industry.

OUTBREAK OF WESTERN CUCUMBER MOSAIC ON SUGAR BEETS¹

HENRY H. P. SEVERIN² and JULIUS H. FREITAG³

SUMMARY

Western cucumber mosaic occurs in the interior regions of California, and not in the coastal fog belt.

The symptoms of western cucumber mosaic on naturally infected sugar beets are: large, pale-yellow chlorotic areas; white or green veinbanding or interveinal chlorosis; blisterlike elevations on younger leaves, often accompanied by distorted midribs and veins or by outward-rolled leaf margins; and deformed or twisted young leaves.

The green peach aphid, *Myzus persicae* (Sulzer), is the most important vector of the virus to sugar beets. The bean or dock aphid, *Aphis rumicis* Linnaeus, rarely transmits the virus to beets.

Systemic infection was obtained with 20 per cent of the beets inoculated by the green peach aphid and 26 per cent of those mechanically inoculated.

INTRODUCTION

A serious outbreak of western cucumber mosaic on sugar beets occurred near Firebaugh and Mendota, in the middle San Joaquin Valley, California, in 1940. Economic and ornamental flowering plants and weeds of many species showed severe symptoms of the disease. Enormous flights of the green peach aphid, *Myzus persicae* (Sulzer), from the plains and foothills of the Inner Coast Range occurred in the beet fields that year. Ladybird larvae and adult beetles devouring aphids were teeming in the beet fields during the spring.

A similar relation between heavy aphid population and an outbreak of another virus disease occurred in 1927. During the spring of that year, aphids were extremely abundant and destroyed most of the pasture vegetation grow-

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ing on the plains and foothills of the Inner Coast Range in the middle and northern San Joaquin Valley, as reported in a previous paper (Severin, 1933).⁴ After the pasture vegetation became wilted and began to dry, swarms of winged aphids flew into the cultivated areas. That year most of the sugar beets in the middle and northern San Joaquin Valley developed symptoms of sugar-beet mosaic; however, no beets showing symptoms of western cucumber mosaic were found. The beet leafhopper, *Eutettix tenellus* Baker, could not have been an important factor in 1927 because its food supply was destroyed in March, before most of the nymphs had acquired the winged stage.

Freitag (1941) reported that ten species of aphids readily transmitted western-cucumber-mosaic virus to 104 of 271 squash plants.

Bennett (1944) described and illustrated yellowish primary lesions on sugar beets after juice inoculation with a strain of cucumber mosaic from sugar beets in the vicinity of Mendota. Systemic infection did not result from such lesions, he reported; but did result from inoculation by *Myzus persicae*.

The present paper reports observations on and experiments with western-cucumber-mosaic virus from 1940 to 1947. The phases investigated were distribution of the disease, methods of transmission of the virus, symptoms of the disease, and recovery of the virus. The symptoms are similar to those of celery calico and common cucumber mosaic on the leaves of sugar beets. To facilitate distinguishing them, a study was made of symptoms of the latter two diseases; this study is reported in the companion paper (Severin, 1948).

GEOGRAPHICAL DISTRIBUTION

The western-cucumber-mosaic virus occurs only in the interior regions of California and not in the coastal fog belt. Entomologists of the Spreckels Sugar Company made surveys of the sugar-beet fields in the southern and northern San Joaquin Valley and in the Sacramento Valley; but they found only an occasional plant to be naturally infected with this disease in 1940 and later years. An examination of the beet fields in the coastal fog belt in the Santa Clara Valley during the 1940 outbreak of the disease failed to show a single beet infected with this cucumber-mosaic virus.

SYMPTOMS

On Experimentally Infected Beets. On leaves of experimentally infected sugar beets in the greenhouse, the sequence of symptoms of western cucumber mosaic is as follows: Beet leaves 7 to 10 days after inoculation show numerous, large, pale-yellow, chlorotic areas, 5 to 10 mm in diameter, each with a circular, chlorotic center enclosed in a narrow dark ring, and with the margins of the areas diffusing into the surrounding green portions of the leaf (fig. 1, *A*). On some inoculated outer leaves, small, chlorotic spots develop among the large yellow areas, and white veinbanding of the midrib and veins occurs (fig. 1, *B*). Later, each yellow area becomes surrounded by a green or yellow ring, and the center becomes purple or pink (fig. 1, *C*). Accompanying these symptoms, veinbanding (fig. 2, *A*) or a reticulate pattern (fig. 2, *B*) may develop. Each circular, chlorotic area becomes necrotic (fig. 1, *D*), and persists after the leaf becomes dry (fig. 2, *C*). Necrosis of the midrib and lateral veins

⁴ See "Literature Cited" for citations, referred to in the text by author and date.

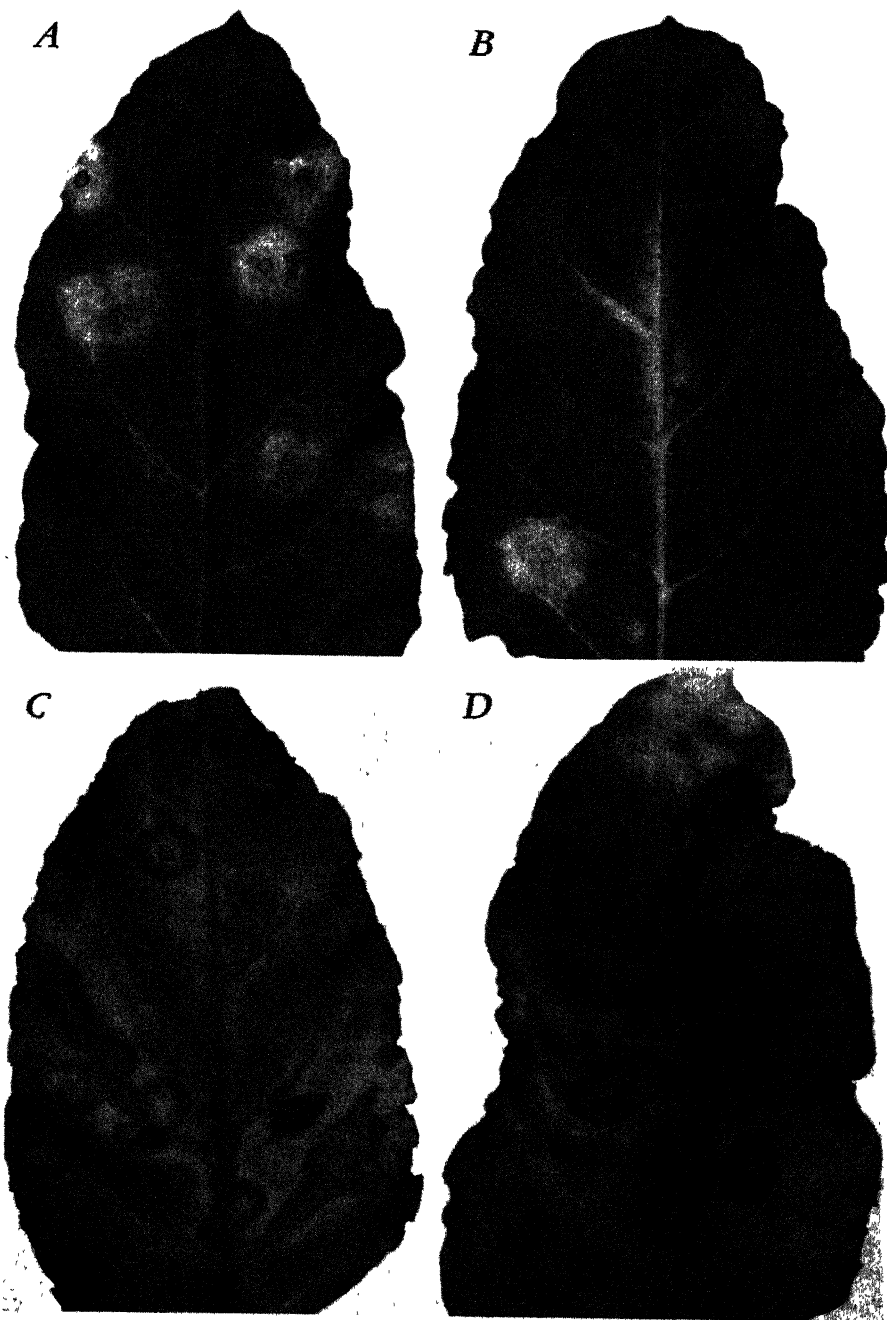


Fig. 1. Symptoms of western cucumber mosaic on leaves of experimentally infected sugar beets: *A*, large, circular, pale-yellow, chlorotic areas with margins diffusing in surrounding green portion; *B*, small and large yellow areas and white veinbanding of midrib and veins; *C*, green or yellow rings enclosing pale-yellow areas with purple or pink centers; *D*, necrotic, circular areas, formerly yellow.

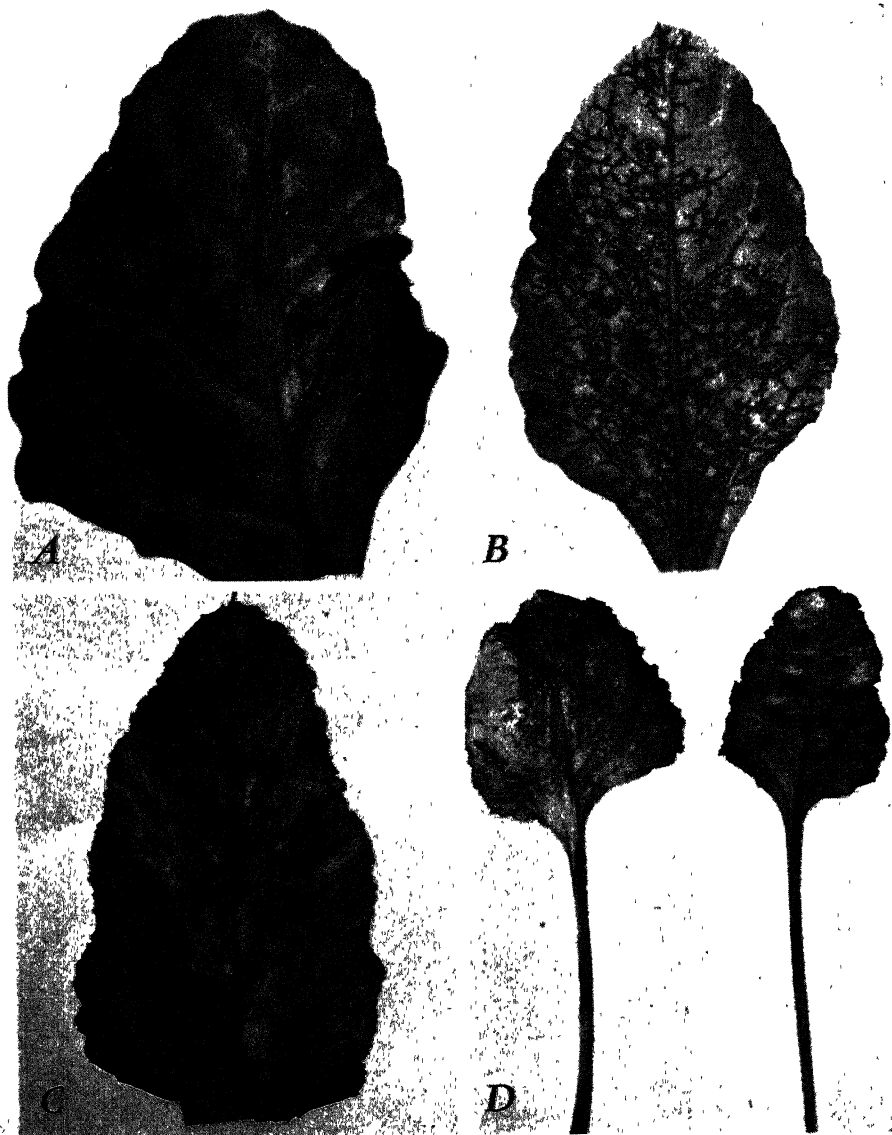


Fig. 2. Symptoms of western cucumber mosaic on leaves of experimentally infected sugar beets: *A*, necrosis and veinbanding; *B*, reticulate pattern; *C*, dried leaf showing necrotic circular areas; *D*, necrosis of petioles, midribs, and rings surrounding chlorotic areas.

occurs (fig. 2, *D*). When infection is systemic, blisterlike elevations develop on the younger leaves. This symptom is like that shown for natural infection in figure 4, and is a reliable indication of systemic infection (page 529). It also helps to distinguish this disease on mechanically inoculated beets from celery calico and common cucumber mosaic, which show somewhat similar chlorotic symptoms. This is further discussed in the companion paper (Severin, 1948).

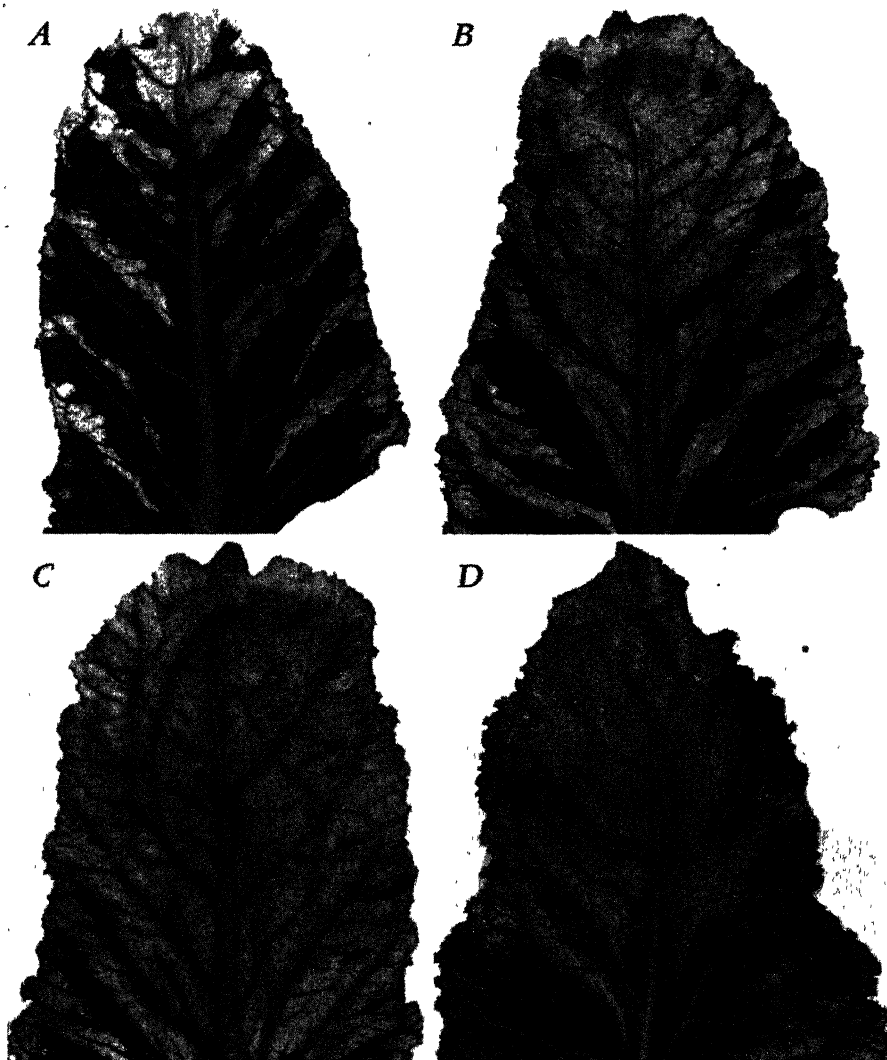


Fig. 3. Symptoms of western cucumber mosaic on young leaves of naturally infected sugar beets: *A*, white or green veinbanding; *B*, chlorosis of apical portion of leaf, interveinal chlorosis, and green veinbanding; *C*, chlorosis of most of the leaf, interveinal chlorosis, and green veinbanding; *D*, chlorosis of upper half of leaf showing interveinal chlorosis, and green, reticulate veinbanding.

On Naturally Infected Beets. Striking symptoms on naturally infected sugar beets, when viewed from the roadside, are the general yellowing of the outer leaves and dark-green intermediate and younger leaves. A closer examination of the outer yellow leaves shows numerous circular, chlorotic areas, like those in experimentally infected beets (fig. 1, *A*); on old outer and on dried leaves these are necrotic. White or green veinbanding or interveinal chlorosis occurs on the intermediate or younger leaves (fig. 3, *A*, *B*, *C*).

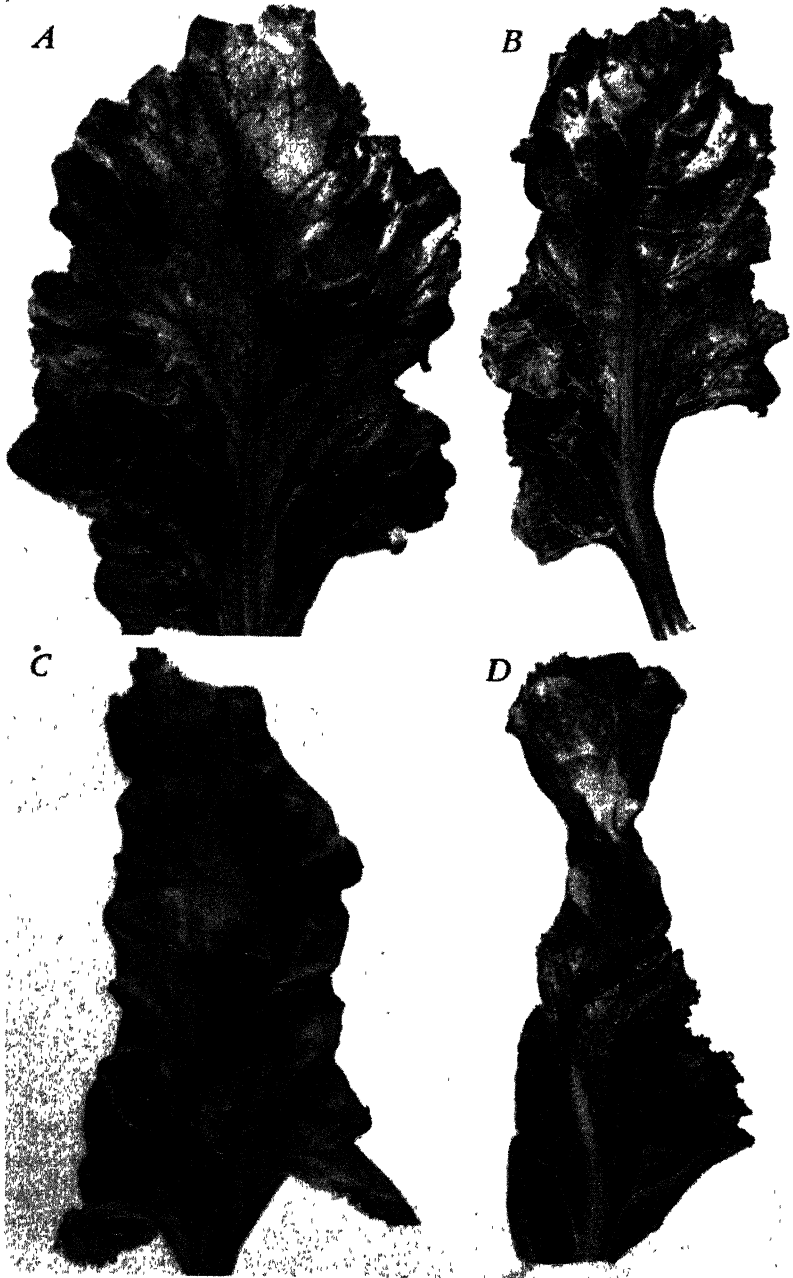


Fig. 4. Symptoms of western cucumber mosaic on leaves of naturally infected sugar beets: *A*, large, green, blisterlike elevations on intermediate leaf; *B*, lower surface of blistered young leaf showing distorted midrib and some of the veins; *C*, outward-rolled margins of blistered young leaf; *D*, deformed young leaf with corkscrew twist. All of the leaves in this figure show chlorosis; but blistering may appear on such intermediate and younger leaves before chlorosis is evident.

Chlorosis begins from the apical portion of the leaf and spreads toward the basal margin (fig. 3, *B*, *C*), with green or reticulate green veinbanding (fig. 3, *D*). A prominent symptom on many of the intermediate and younger leaves is numerous, dark-green, blisterlike elevations (fig. 4, *A*), which may persist after chlorosis of the leaf occurs. The midribs and veins of blistered leaves are frequently distorted (fig. 4, *B*). The margins of the younger, blistered leaves may be rolled outward (fig. 4, *C*). The young leaves may be deformed (fig. 4, *C*), sometimes with a corkscrew twist (fig. 4, *D*).

TRANSMISSION AND RECOVERY OF THE VIRUS

Aphid Vectors. Several species of aphids were tested as vectors of western-cucumber-mosaic virus; the methods used have been described previously (Severin and Freitag, 1938). The following species, reported to occur on beets under natural conditions (Gillette and Palmer, 1934; Patch, 1938), proved to be vectors:

Cotton or melon aphid, *Aphis gossypii* (Glover)

Bean or dock aphid, *Aphis rumicis* Linnaeus

Green peach aphid, *Myzus persicae* (Sulzer)

The green peach aphid is the most important vector of the virus to sugar beets. The bean aphid rarely transmits the virus to beets. The cotton aphid is an efficient vector of the virus to melons and cucumbers. The potato aphid, *Macrosiphum solanifolii* (Ashmead), which also occurs on beets, has not been tested as a vector of this disease.

Mechanical Inoculation Compared with Aphid Transmission. Except where otherwise indicated, the virus was transmitted by mechanical inoculation with the carborundum method (Rawlins and Tompkins, 1936); the expressed sap from sugar beets and other host plants was used.

Mechanical inoculation was compared with the transmission of the virus by the green peach aphid, *Myzus persicae*. The formation of blistering on the youngest leaves of beet seedlings was used as the criterion of systemic infection (see next paragraph). A large population of aphids was reared on 7 sugar beets showing blisterlike elevations on the youngest leaves. Five lots of 20 aphids each were transferred from each diseased plant to healthy beet seedlings, 1 lot to a seedling. The virus extract from each infected plant, on which the aphids had fed, was also inoculated mechanically into 5 healthy beet seedlings. Blistering on the youngest leaves developed on 7 of 35 beets, or 20 per cent, inoculated by the green peach aphid; and on 9 of 35 beets, or 26 per cent, mechanically inoculated. Contrary to Bennett's (1934) results with juice inoculations, previously mentioned, the type of infection was systemic with mechanical inoculation as well as with aphid transmission.

Recovery of the Virus. When the outer leaves of large beets were inoculated, symptoms developed on them; but blistering appeared on the youngest leaves in only about one fourth of the plants. When such blistering did appear, the virus was recovered from the outer, intermediate, and inner leaves of naturally and experimentally infected sugar beets and transferred to healthy sugar beets by mechanical inoculation. When it did not appear, the virus was recovered only from the outer, inoculated leaves showing symptoms. Thus blistering proved to be a reliable criterion of systemic infection.

HOST RANGE, NATURAL INFECTION

The following economic plants were demonstrated to be naturally infected with the virus:

Chenopodiaceae:

- Sugar beet, *Beta vulgaris*
- Garden or red beet, *Beta vulgaris*
- Swiss chard, *Beta vulgaris* var. *cicla*
- Spinach, *Spinacia oleracea*

Compositae:

- Lettuce, *Lactuca sativa*

Cucurbitaceae:

- Honey Dew melon, *Cucumis melo* var. *inodorus*
- Cucumber, *Cucumis sativus*
- West Indian or bur gherkin, *Cucumis anguria*

Solanaceae:

- Tomato, *Lycopersicon esculentum*

Umbelliferae:

- Celery, *Apium graveolens* var. *dulce*

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SYMPTOMS OF ADDITIONAL CUCUMBER-MOSAIC VIRUSES ON SUGAR BEETS¹

HENRY H. P. SEVERIN²

SUMMARY

Celery calico occurs on celery and on some other host plants in the interior regions and in the fog belt of California; but has not yet been found on sugar beets here. In mechanically inoculated sugar beets, the type of infection is local.

The virus of common cucumber mosaic is not known to occur naturally in California. In mechanically inoculated sugar beets, the type of infection is local.

Symptoms of these two diseases on mechanically inoculated sugar beets resemble those of western cucumber mosaic, which does occur on this host plant in California. In all three, chlorotic areas, veinbanding, and necrosis occur. The first symptom of celery calico—large, irregular, pale-green areas on the leaves—serves to distinguish it from common cucumber mosaic. Common cucumber mosaic can be distinguished from both celery calico and western cucumber mosaic by the first symptom—small, white dots with pinpoint necrotic centers; later by small, rust-colored necrotic centers in circular, chlorotic areas; still later by holes in some leaves caused by the dropping out of the necrotic centers. Neither celery calico nor common cucumber mosaic show, in mechanically inoculated beets, the blisterlike elevations that characterize systemic infection with western cucumber mosaic.

INTRODUCTION

The symptoms of celery calico and common cucumber mosaic closely resemble those of western cucumber mosaic. To facilitate distinguishing them, studies were made of the symptoms of all three diseases in experimentally infected sugar beets. This paper describes celery-calico and common-cucumber-mosaic symptoms on that host plant. Symptoms of western cucumber mosaic on experimentally and naturally infected sugar beets are described in the first paper of this issue (Severin and Freitag, 1948).

A number of references appear in the literature concerning the transmission of common cucumber mosaic or strains of this virus to beets (*Beta vulgaris*). Johnson (1930)³ inoculated Crosby's Egyptian garden beet with common cucumber mosaic types 1 and 2, and necrotic rings $\frac{1}{8}$ inch in diameter developed on the rubbed leaves 10 days after inoculation. These rings increased to $\frac{1}{4}$ inch in diameter, at which time they were composed of alternate bands of necrotic and red tissues.

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² Entomologist in the Experiment Station.

³ See "Literature Cited" for citations, referred to in the text by author and date.

Hoggan (1933) demonstrated that local symptoms resulted from mechanical inoculation of sugar-beet leaves with the common-cucumber-mosaic virus, but systemic infection was not secured. When the green peach aphid (*Myzus persicae*) and the potato aphid (*Macrosiphum solanifolii*) were confined to a single sugar-beet leaf, local lesions developed. The virus appears unable to pass from a single infected leaf of this host to other parts of the plant. When the infective aphids were allowed to feed freely on the foliage of young sugar beets, systemic infection was readily obtained; she suggests that this may possibly have resulted from a direct introduction of virus at or near the growing point of the shoot, the virus perhaps multiplying at this point and forming a source of infection for all subsequent growth.

Price (1940), in his table on species of plants tested for susceptibility to six viruses, lists *Beta vulgaris* as susceptible to the cucumber-mosaic virus (*Marmor cucumeris* H. var. *vulgare* H., *judicis* H., and *vignae* H.; mosts tests with the ordinary strain, *vulgare*).

MATERIALS AND METHODS

The common-cucumber-mosaic virus was kindly sent to me by James Johnson, University of Wisconsin. The original source of the celery-calico virus was naturally infected celery obtained near Milpitas in the Santa Clara Valley. The viruses were maintained by repeated mechanical inoculation of various host plants; and also the virus extract was kept overwinter in cold storage at -18°C .

The method of mechanical inoculation used is that described by Rawlins and Tompkins (1936). Shortly after inoculation, the carborundum and the inoculum were washed from the leaves with water. No tests of insect transmission were included in these experiments.

CELERY CALICO

Celery calico has been found on celery in all of the large celery districts in California (Severin and Freitag, 1938). The disease occurs in the interior regions and in the fog belt of the state. The distribution of celery calico on naturally infected perennial delphiniums has been reported in a previous paper (Severin, 1942*a*). Other naturally infected host plants include larkspurs (Severin, 1942*b*) and pansies and violas (Severin, 1947). Up to the present time, no attempt has been made to find sugar beets naturally infected with this disease. It could easily pass unnoticed because the symptoms appear only on the inoculated leaves.

Symptoms. The first symptom of celery calico on the leaves of sugar-beet seedlings, 3 to 9 days after inoculation (4 to 15 days on large beets), is large, irregular, pale-green areas (fig. 1, *A*), which diffuse into the green tissue. (This symptom serves to distinguish this disease from common cucumber mosaic.) Later, these become circular yellow areas, 11 to 15 mm in diameter, each with a pale chlorotic center (fig. 1, *B*). Chlorotic veinbanding of a portion of the midrib and some of the veins may occur (fig. 1, *B*). Chlorosis spreads gradually over the entire leaf. Irregular green, later chlorotic, rings surround the circular yellow areas (fig. 1, *C*); these enlarge, coalesce, and become irregular in shape (fig. 1, *D*). The fused rings become necrotic (fig. 2, *A*), usually

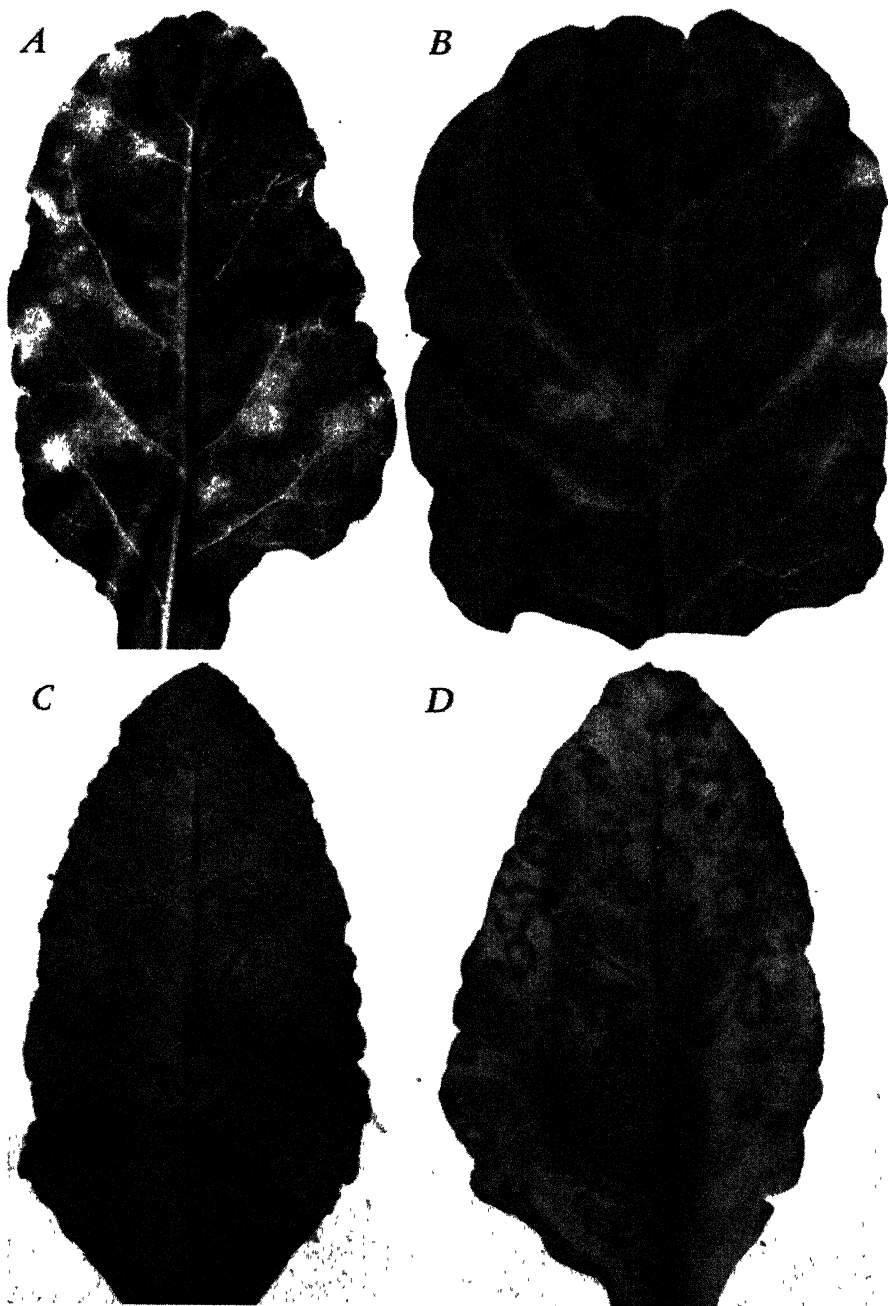


Fig. 1. Symptoms of celery calico on experimentally infected sugar beets: *A*, circular, chlorotic areas with margins diffusing in green areas; *B*, circular, chlorotic areas, some showing pale, circular center, veinbanding of portion of midrib and some of the veins; *C*, irregular, green, later yellow, rings surrounding pale-green areas which become chlorotic; *D*, yellow rings coalescing.

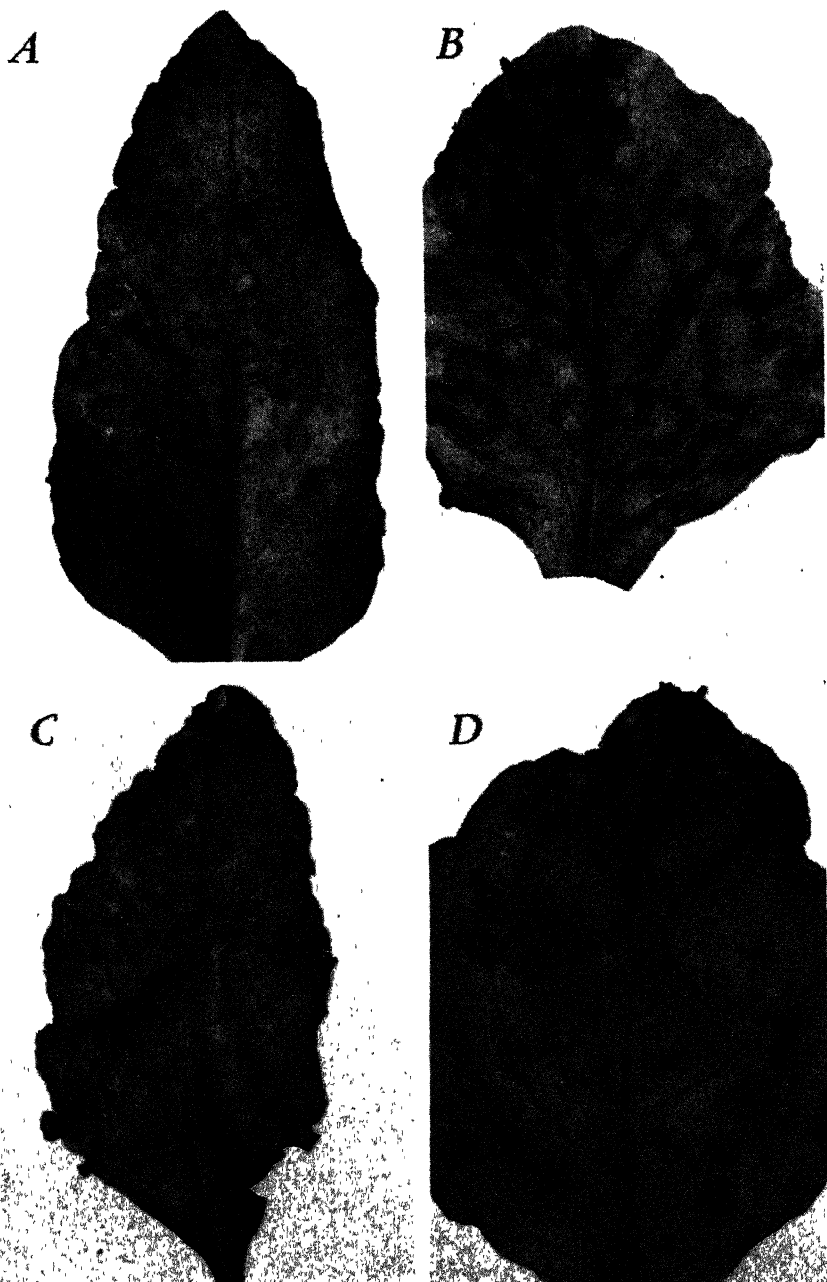


Fig. 2. Symptoms of celery calico on leaves of experimentally infected sugar beets: *A*, fused, necrotic rings; *B*, necrosis of apical portion of leaf, midrib, and veins, and necrotic rings still evident in dried portion of leaf; *C*, yellowing of upper half of leaf showing necrotic rings; *D*, necrosis of rings and enclosure. Note that the chlorotic areas in this disease lack the small rust-colored centers that are often present in common cucumber mosaic (see fig. 5, *A*).

after the leaf becomes yellow (fig. 2, *B, C*); and finally the entire circular areas become necrotic (fig. 2, *D*) and are still evident on the dried leaves (fig. 2, *B*).

Recovery of the Virus. The type of infection by mechanical inoculation, was local, not systemic. The virus was recovered only from the inoculated leaves and transferred to healthy sugar beets, cucumbers, celery, and Turkish tobacco (*Nicotiana tabacum*) by mechanical inoculation.

COMMON CUCUMBER MOSAIC

Common cucumber mosaic is not known to occur on any host plant in California.

Symptoms. The first symptom of common cucumber mosaic on sugar-beet leaves, 4 to 9 days after inoculation, is numerous, white dots (fig. 3, *A*), each with a pinpoint, necrotic center. These dots gradually enlarge (fig. 3, *B*) and each retains the pinpoint, necrotic center. The white, circular areas fuse (fig. 4, *A*). Green rings, 3 to 8 mm in diameter, surround pale, chlorotic areas, with a small, circular central area (fig. 4, *B*) and a necrotic center. Within the next 2 or 3 days the green rings become yellow, enclosing chlorotic areas, each with a small central, circular area with a necrotic center (fig. 5). Sometimes concentric green and yellow rings surround chlorotic areas, each with a rust-colored, necrotic center. Frequently the rings coalesce (fig. 5, *C, D*). The chlorotic areas surrounded by rings may become brown, drop out, and leave

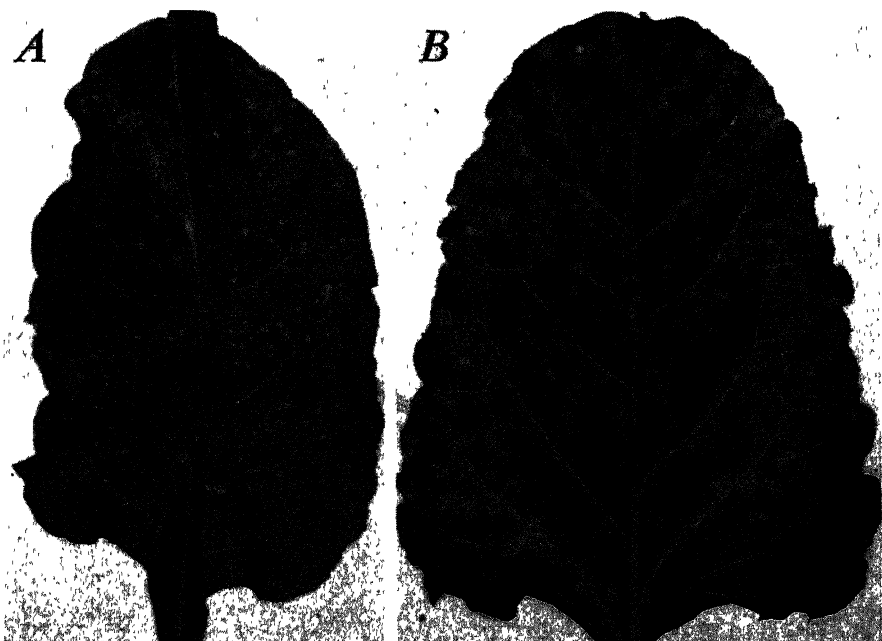


Fig. 3. Symptoms of common cucumber mosaic on leaves of experimentally infected sugar beets: *A*, numerous white dots; *B*, dots enlarged to form white, circular areas. These dots, the first symptom to appear in this disease, do not occur in celery calico or in western cucumber mosaic.

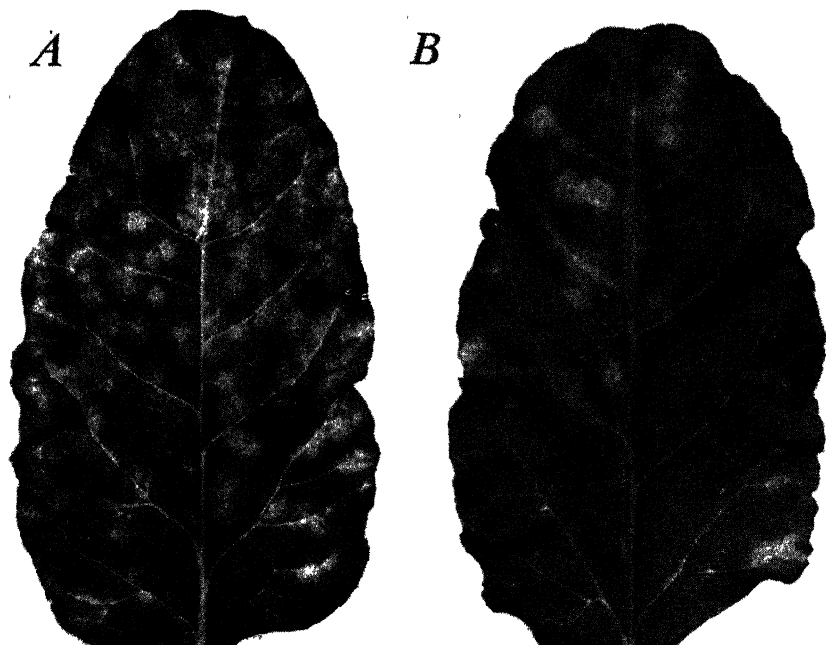


Fig. 4. Symptoms of common cucumber mosaic on leaves of experimentally infected sugar beets: *A*, fusion of white circular areas; *B*, green rings surrounding chlorotic areas, each with a small, central, circular spot.

holes in the leaf. Yellow veinbanding along part of the midrib and lateral veins develops (fig. 5). Sometimes forked chlorotic tissue extends from the rings (fig. 5, *A*, *B*). In the later stage of the disease, necrosis of the rings occurs, and the rings are still evident on the dried leaves.

The symptoms that are useful in distinguishing this disease from celery calico and western cucumber mosaic are the small white dots with pinpoint necrotic centers (fig. 3, *A*)—the first symptom to appear; in a somewhat later stage, the small rust-colored necrotic centers of some chlorotic areas (fig. 5, *C*); and still later the holes in some leaves caused by the dropping out of the necrotic centers.

Recovery of the Virus. The virus was recovered only from the inoculated leaves and transferred to healthy sugar beets, cucumbers, and celery by mechanical inoculation. The type of infection was local and not systemic, agreeing with the results Hoggan (1933) obtained with mechanical inoculation of this virus.

Fig. 5. Symptoms of common cucumber mosaic on leaves of experimentally infected sugar beets: *A*, yellow rings with large, necrotic, rust-colored centers and yellow veinbanding; *B*, fusion of rings, some with two necrotic centers, and yellow veinbanding of midrib and lateral veins; *C*, *D*, fusion of many necrotic rings with 1 to 5 necrotic centers, and veinbanding.

A



B



C



D



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APHID TRANSMISSION OF MILD MOSAIC VIRUS OF ANNUAL STOCK¹

HENRY H. P. SEVERIN² and C. M. TOMPKINS³

SUMMARY

Five species of aphids, tested in lots of 20, were demonstrated to be vectors of mild-mosaic virus of annual stock; these are:

Bur clover or cowpea aphid, *Aphis medicaginis* Koch

Cabbage aphid, *Brevicoryne brassicae* Linnaeus

Artichoke aphid, *Myzus braggi* (Gillette)

Green peach aphid, *Myzus persicae* (Sulzer)

Turnip or false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis)

Of these, only the turnip aphid breeds on annual stock plants under natural conditions. It causes pale-green circular areas around the mouth-part punctures, and, when abundant, dwarfing and yellowing or blanching of the flowers.

The turnip aphid failed to transmit the virus to nine varieties of healthy cauliflower.

Several infections were obtained with single turnip and green peach aphids fasted for 2 hours, fed ½, 1, 2, 5, or 10 minutes on leaves from infected stock plants, and then transferred to healthy plants, 1 aphid per plant. No infections were obtained with several hundred turnip, green peach, and cabbage aphids tested singly without fasting and with longer periods on infected stock plants.

In tests on retention of the virus, turnip aphids, fasted for 30 minutes, then fed singly 5 or 10 minutes on mild-mosaic-infected annual stock and 5 or 10 minutes on 5 or 6 successive healthy stock plants, produced infections only in the first healthy plant.

Lots of 20 turnip aphids had lost their infectivity by the fourth day after transfer from an infected to a previously healthy stock plant. If allowed to remain on the plant from 7 to 13 days, however, the aphids were able to recover the virus from the plant they had inoculated; this was long before symptoms appeared. The incubation period of the disease in the original inoculated plants varied from 16 to 22 days.

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INTRODUCTION

In 1930, commercial growers of annual stock (*Mathiola incana* var. *annua*) observed a serious disease that caused breaking in the color of flower petals. Field infection was severe from 1931 to 1948. The disease destroyed the value of the plants for cut flowers and reduced seed production. The trouble was identified as a virus disease (Tompkins, 1934).^{*} Tompkins (1939) named it mild mosaic of annual stock, and distinguished it from severe mosaic, another virus disease of this host, which causes even more severe breaking and occurs under field conditions in San Pablo, California. He described the symptomatology, transmission, host range, and properties of the two viruses.

Breaking in the color of petals has been reported to result from mechanical inoculation with several crucifer mosaic viruses, in addition to the two stock viruses—namely, turnip mosaic (Tompkins, 1938; Chamberlain, 1939), cabbage black ring (Tompkins, Gardner, and Thomas, 1938), Chinese cabbage mosaic (Tompkins and Thomas, 1938), cabbage mosaic (Larson and Walker, 1938), and horseradish mosaic (Tompkins, 1939). Investigators in various countries have reported breaking in the color of petals or mosaic disease in annual stock, without identifying the causal virus (see Tompkins, 1939).

Annual stock has been demonstrated to be naturally infected with sugar-beet curly top in California (Severin, 1934), and with an unidentified disease resembling California aster yellows; but these do not cause breaking.

In connection with the investigation of other phases of mild and severe mosaics of annual stock by Tompkins (1939), experiments were undertaken in 1934 on various phases of aphid transmission. Tompkins reported early results obtained by the senior author: the turnip or false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis), the cabbage aphid, *Brevicoryne brassicae* Linnaeus, and the green peach aphid, *Myzus persicae* (Sulzer), were demonstrated to be vectors of both viruses. The turnip aphid breeds on stock under natural conditions, the other two do not.

The present paper reports further results of experiments on aphid transmission of mild mosaic of annual stock; phases investigated include transmission of the virus by several aphid species from infected stock plants to healthy stock and cauliflower plants, comparison of transmission of the virus by mechanical inoculation with that by three species of aphids, transmission of the virus by single aphids and during short feeding periods, the retention of the virus, and loss and recovery of infectivity by aphids. Symptoms on foliage and flowers caused by feeding of noninfective aphids were studied and differentiated from those caused by the mild-mosaic virus.

MATERIALS AND METHODS

The virus causing mild mosaic of annual stock was obtained at Montara, San Mateo County. Plants of the Fiery Blood Red variety of annual stock and the February variety of cauliflower grown from seeds were used in all experiments. Methods used in aphid-transmission experiments were similar to those used previously (Severin and Freitag, 1938). The carborundum method (Rawlins and Tompkins, 1936) was used in mechanical inoculations.

^{*} See Literature Cited for citations, referred to in the text by author and date.

DISTRIBUTION

Annual stock naturally infected with mild mosaic is generally distributed on seed farms and in home gardens in the coastal districts of California. Stock plants showing breaking in the color of the petals were obtained at Davis and the virus was recovered and transferred by means of the turnip aphid, *Rhopalosiphum pseudobrassicae* (Davis) (plate 1), to healthy stock.

SYMPTOMATOLOGY

Induced by Feeding of Aphids. Noninfective and infective turnip aphids, *Rhopalosiphum pseudobrassicae*, produced pale-green circular areas (plate 2, *B*) around the mouth-part punctures on the leaves. The flowers of stock plants which had large populations of aphids (plate 1) were dwarfed, and frequently the tips of the petals were yellow or white; sometimes most or all of the petals were yellowish green (plate 2, *C*).

Mild Mosaic. Tompkins (1934, 1939) has described the symptoms of mild mosaic on annual stocks. The more important symptoms are briefly reviewed here for comparison with the symptoms induced by the feeding of aphids.

The first symptom of mild mosaic on the younger leaves of annual stock appears 2 to 3 weeks after inoculation, as a clearing of the veins and veinlets (plate 3, *B, C, D*) followed by mottling consisting of pale and dark-green areas (plate 3, *E*). These symptoms rarely occur on naturally infected stock plants. Sometimes the apical leaves are distorted, curled, and puckered. Infected plants are slightly or severely stunted with shortened internodes.

A striking flower symptom is breaking in color of the petals (plate 4, *B, C*). Sometimes the cluster of flowers at the apical end of the stems is apparently normal, while the lower racemes show breaking; usually, however, all racemes show breaking. A reduction of the number and size of the seed pods occurs.

APHID TRANSMISSION OF VIRUS

By Vectors That Do Not Breed on Stock under Natural Conditions. No intensive investigations were undertaken on aphid vectors which do not breed on annual stock plants under natural conditions.

Bur clover or cowpea aphids, *Aphis medicaginis* Koch, collected on lamb's-quarters (white pigweed) (*Chenopodium album*) and on rough pigweed (*Amaranthus retroflexus*) growing among annual stock plants near Montara, were transferred from these weeds to stock infected with mild mosaic. A high mortality of the aphids occurred on annual stock. Three of 6 lots of 20 aphids each, changed from mosaic to healthy annual stock plants, caused infection.

Artichoke aphids, *Myzus braggi* (Gillette), collected on artichokes near El Granada, were transferred to mosaic-infected annual stock, and then to healthy annual stock plants. Three lots of 20 aphids produced 2 infections.

Green peach aphids, *Myzus persicae*, collected on sugar beets, were transferred to naturally infected stock plants and thence to healthy stock and cauliflower plants. The results are given in table 1. The cabbage aphid, *Brevicoryne brassicae*, was also demonstrated to be a vector of this virus.

By a Vector That Breeds on Stock under Natural Conditions. Whenever a large population of the turnip aphid was found on naturally infected

annual stock plants showing breaking in the flowers, lots of 20 aphids were transferred from the mosaic to healthy annual stock plants and to healthy cauliflower seedlings. This aphid transmitted the virus to 60 per cent of the stock plants inoculated, but to none of the cauliflower plants (table 1).

From Experimentally Infected to Healthy Annual Stock Plants and Varieties of Cauliflower. Lots of 20 infective turnip aphids were transferred from

TABLE 1
TRANSMISSION OF MILD-MOSAIC VIRUS FROM NATURALLY INFECTED
TO HEALTHY ANNUAL STOCK BY TWO SPECIES OF APHIDS

Aphid species and district in which infected plants were found	Annual stock		Cauliflower	
	Inoculated	Infected	Inoculated	Infected
Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i>:				
Alameda County:				
Berkeley.....	10	2	10	0
Berkeley.....	6	5	6	0
San Francisco County:				
San Francisco...	6	2	6	0
San Mateo County:				
Burlingame.....	12	10	12	0
Montara.....	10	8	10*	0
Montara.....	10	8	10	0
Montara.....	10	3	10	0
Yolo County:				
Davis.....	12	6	12	0
Davis.....	6	5	6	0
Total.....	82	49	82	0
Percentage.....	..	60	..	0
Green peach aphid, <i>Myzus persicae</i>:				
Alameda County:				
Berkeley..	5	4	5	0
Berkeley.....	5	3	5	0
San Francisco County:				
San Francisco...	5	3	5	0
San Mateo County:				
Burlingame...	5	2	5	0
Montara.....	5	2	5	0
Montara.....	5	2	5	0
Montara.....	5	2	5	0
Yolo County:				
Davis.....	5	1	5	0
Davis.....	5	1	5	0
Total.....	45	20	45	0
Percentage.....	..	45	..	0

experimentally infected annual stock to healthy annual stock plants and nine varieties of cauliflower. Table 2 shows that 51 per cent of the annual stock plants, but none of the cauliflower plants, were infected. Each cauliflower plant was again inoculated with a lot of 20 infective false cabbage aphids, and again no infections occurred. The fact that no infection of cauliflower was obtained with the mild-mosaic virus of annual stock serves to differentiate this virus from the cauliflower-mosaic virus, a conclusion which Tompkins (1937) reached from his results with mechanical inoculation.

Comparison of Aphid Transmission and Mechanical Inoculation. The transmission of the virus from experimentally infected to healthy stock plants by mechanical inoculation was compared with transmissions by three species of aphids. The virus extract from infected plants upon which the aphids had

TABLE 2
TRANSMISSION OF VIRUS FROM STOCKS EXPERIMENTALLY INFECTED
WITH MILD MOSAIC TO HEALTHY ANNUAL STOCK AND
CAULIFLOWER BY THE TURNIP APHID

Mosaic-infected stock plant no.	Annual stock		Cauliflower		
	Inocu- lated	Infected	Variety	Inocu- lated	Infected
1.	5	3	Danish Perfection	5	0
2	5	0	Dryweather Danish Giant.	5	0
3	5	1	Extra Early Dwarf Erfurt.	5	0
4	5	2	Hartmans Special.	5	0
5	5	1	Hartmans Special Medium	5	0
6	5	1	Late Pearl.	5	0
7	5	4	February 759*	5	0
8	5	5	Early March 713*	5	0
9	5	5	Mission Special 4577*	5	0
Total	45	22		45	0
Percentage		49			0

* Grown from seed from Ferry-Morse Seed Co., San Francisco.

TABLE 3
COMPARISON OF TRANSMISSION OF MILD-MOSAIC VIRUS OF ANNUAL
STOCK BY MECHANICAL INOCULATION AND BY
THREE SPECIES OF APHIDS

Number of plants from which virus was recovered	Mechanical inoculation			Aphid transmission			
	Plants inocu- lated	Plants infected	Per cent infected	Aphid species	Plants inocu- lated	Plants infected	Per cent infected
5	25	20	80	Cabbage aphid, <i>Brevicoryne brassicae</i>	25	1	4
10	50	7	14	Turnip aphid, <i>Rhopalosiphum pseudobrassicae</i>	50	22	44
5	25	15	60	Green peach aphid, <i>Myzus persicae</i>	25	13	52

fed was inoculated into healthy plants. The results obtained are given in table 3. The turnip aphid was more efficient in transmitting the virus than was mechanical inoculation, the cabbage and green peach aphids less so.

By Single Aphids. An attempt was made to determine the efficiency of virus transmission by three species of mature aphids fed 1 day on mosaic stock plants, then each fed singly on a healthy stock plant. Equal numbers of winged and wingless aphids were used. Not a single infection was obtained with 300 turnip aphids, 200 cabbage aphids, and 150 green peach aphids.

TABLE 4
SHORT FEEDING TIME ON MILD-MOSAIC STOCK PLANTS
BY SINGLE WINGLESS APHIDS OF TWO SPECIES

Aphid species	Feeding time, minutes									
	0.5		1		2		5		10	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
Turnip aphid, <i>Rhopalosiphum pseudobrassicæ</i>	5	1	5	0	5	0	5	1	5	0
Green peach aphid, <i>Myzus persicae</i>	5	2	5	1	5	1	5	0	5	1

TABLE 5
RETENTION OF MILD-MOSAIC VIRUS OF ANNUAL
STOCKS BY TWO SPECIES OF APHIDS

Aphid species and test no.	Number of aphids on first plant	Number of annual stock					
		Inocu- lated 1st day	Infected 1st day	Inocu- lated 2d day	Infected 2d day	Inocu- lated 3d day	Infected 3d day
Turnip aphid, <i>Rhopalosiphum pseudobrassicæ</i>:							
Test 1.....	5	12	2	12	0	12	0
Test 2.....	10	6	1	6	0	6	0
Test 3.....	20	7	4	7	0	7	0
Test 4.....	20	6	3	6	0	6	0
Test 5.....	20	6	1	6	0	6	0
Test 6.....	20	5	4	5	0	5	0
Test 7.....	20	5	1	5	0	5	0
Test 8.....	20	5	1	5	0	5	0
Test 9.....	20	4	1	4	0	4	0
Total		56	18	56	0	56	0
Percentage.....	32	..	0	..	0
Green peach aphid, <i>Myzus persicae</i>:							
Test 1.....	20	5	4	5	0	5	0
Test 2.....	20	5	3	5	0	5	0
Test 3.....	20	5	3	5	0	5	0
Test 4.....	20	5	3	5	0	5	0
Test 5.....	20	5	2	5	0	5	0
Test 6.....	20	5	2	5	0	5	0
Test 7.....	20	5	2	5	0	5	0
Test 8.....	20	5	2	5	0	5	0
Test 9.....	20	5	2	5	0	5	0
Test 10.....	20	5	2	5	0	5	0
Test 11.....	20	5	1	5	0	5	0
Test 12.....	20	5	1	5	0	5	0
Test 13.....	20	5	1	5	0	5	0
Test 14.....	20	5	1	5	0	5	0
Total	70	29	70	0	70	0
Percentage.....	41	..	0	..	0

Because of the failure of the single-aphid tests, further tests of the turnip and green peach aphids were made with a different technique. Noninfective, wingless aphids of these two species were fasted in a phial for 2 hours. Five lots of 5 aphids each were transferred from the phial to a leaf from an infected stock plant and fed $\frac{1}{2}$, 1, 2, 5, or 10 minutes; and then each aphid was transferred to a healthy plant. With the turnip aphid two infections were obtained, at $\frac{1}{2}$ - and 5-minute feeding times, and with the green peach aphid five infections at $\frac{1}{2}$ -, 1-, 2-, and 10-minute feeding times (table 4).

TABLE 6
RETENTION OF MILD-MOSAIC VIRUS OF ANNUAL STOCK BY SINGLE
TURNIP APHIDS, IN SHORT FEEDING PERIODS

Time on diseased annual stock, minutes	Penetration time of stylets in first healthy annual stock, minutes	Feeding time on successive healthy annual stock, minutes*					
		5	10	10	10	10	10
5	7	+	-	-	-	-	-
5	5	+	-	-	-	-	-
10	4	..	+	-	-	-	-
10	3	..	+	-	-	-	-
10	2	..	+	-	-	-	-
Total	4.2	2+	3+	5-	5-	5-	5-

* The plus sign (+) indicates the production of the disease, and the minus sign (-) shows that no disease resulted.

Effect on Flowers. Tests were made to determine the effect on the flowers of infecting annual stock plants at various stages of flower-bud development; the turnip aphid was used for transmission. On plants infected when flower buds were large, the apical cluster of flowers sometimes showed no breaking in color of the petals, but the lower flowers developed breaking. Plants infected before flower buds were visible or when buds were small showed breaking in all flowers.

RETENTION OF VIRUS

By Varying Numbers of Aphids. The retention of the virus was determined for the turnip and the green peach aphids. In the preliminary work, lots of 5 and 11 aphids were transferred daily for 20 days to successive healthy plants, and the aphids remained on the last healthy annual stock plant for one week. No infections were obtained after the first day.

In later work (table 5) lots of 20 infective aphids reared on mild-mosaic stock plants were transferred daily for 3 days to successive healthy stock plants. Both species of aphids transmitted the virus from diseased to healthy annual stock during the first day, but none of the lots tested caused infection the second or third days.

By Single Aphids in Short Feeding Times. Noninfective, mature, wingless, turnip aphids were fasted for 30 minutes in a moist chamber, then were transferred singly to annual stock infected with mild mosaic for a feeding time of 5 or 10 minutes, and then to 5 or 6 successive healthy annual stocks for a feeding time of 5 or 10 minutes on each plant. Table 6 shows that 5 aphids tested singly transmitted the virus to only the first annual stock plant. Sixty-six aphids failed to transmit the virus (not included in table 6).

LOSS AND RECOVERY OF INFECTIVITY BY TURNIP APHIDS

An experiment was conducted to determine whether the turnip aphid could recover the mild-mosaic virus from annual stock plants before breaking in color of petals appeared. A large population of aphids reared on mild-mosaic-

TABLE 7
LOSS AND RECOVERY OF INFECTIVITY BY TURNIP APHID ON
STOCK PLANTS INOCULATED WITH MILD-MOSAIC VIRUS

Original plant number	Results* when a lot of 20 aphids was transferred from the first inoculated plant to a second healthy plant on:											Days to color breaking on petals of original plants
	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	
1	-	-	-	-	+	-	-	+	-	+	+	37
2	-	-	-	-	+	-	-	-	-	+	+	38
3	-	-	-	+	-	-	+	+	-	-	+	40
4	-	-	-	-	-	-	-	-	+	-	+	37
5	-	-	-	-	-	-	+	-	-	+	+	44
6	-	-	-	-	+	-	-	-	-	+	+	44
7	-	-	-	-	-	+	-	-	-	-	+	44
8	-	-	-	-	+	-	+	-	-	-	+	46
9	-	-	-	-	-	-	-	+	-	+	-	38
10	-	-	-	-	+	-	-	-	-	+	+	40
11	-	-	-	-	-	+	-	-	+	+	-	39
12	-	-	-	-	+	-	-	-	-	+	-	45
13	-	-	-	+	-	-	-	+	+	-	-	34
14	-	-	-	-	-	-	+	+	+	-	-	38
15	-	-	-	-	-	-	-	-	+	+	-	38
16	-	-	-	-	+	-	-	-	-	-	+	39
17	-	-	-	-	-	-	-	-	-	+	-	40
18	-	-	-	-	-	-	-	-	+	-	+	41
19	-	-	-	-	-	-	+	-	+	-	-	46
20	-	-	-	-	+	-	+	+	-	-	-	39
21	-	-	-	+	-	-	-	+	-	-	-	30
22	-	-	-	-	-	+	-	-	-	-	-	42
23	-	-	-	-	-	-	-	-	+	-	+	41
24	-	-	-	-	+	+	-	-	-	-	+	43
25	-	-	-	-	+	+	-	-	-	-	+	44
26	-	-	-	-	+	+	-	-	-	-	-	40
27	-	-	-	-	-	+	-	-	-	+	+	43
28	-	-	-	-	-	+	-	-	-	-	-	44
29	-	-	-	-	-	+	+	-	-	-	+	47
30	-	-	-	-	-	+	+	-	-	+	+	48
Total +	0	0	0	3	11	10	8	7	8	12	17	
Total -	30	30	30	27	19	20	22	23	22	18	13	

* The plus sign (+) indicates the production of the disease, and the minus sign (-) shows that no disease resulted.

infected stock plants was transferred to 36 large healthy stock plants for 3 days. Daily from the fourth to the fourteenth day, one lot of 20 of these aphids was transferred from each of the plants so inoculated to a healthy stock plant, where it was left for 3 days; then the plant was fumigated to kill the aphids.

As table 7 shows, no infections were obtained with any of the lots transferred from the fourth to the sixth day; the aphids had lost their infectivity after the first transfer. Infections were obtained with some lots transferred

on the seventh and later days, however; so that the aphids must have recovered the virus from the first inoculated plants. Breaking did not appear on these plants until 30 to 48 days after inoculation. Tompkins (1939) reported an incubation period of 16 to 22 days on mechanically inoculated plants. The virus was not recovered from 6 plants (not included in table 7) during the entire period.

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Plate 1. Heavy population of turnip aphids, *Rhopalosiphum pseudobrassicae*, on annual stock. White specks are molted skins. (Davis, California, August 3, 1934.)

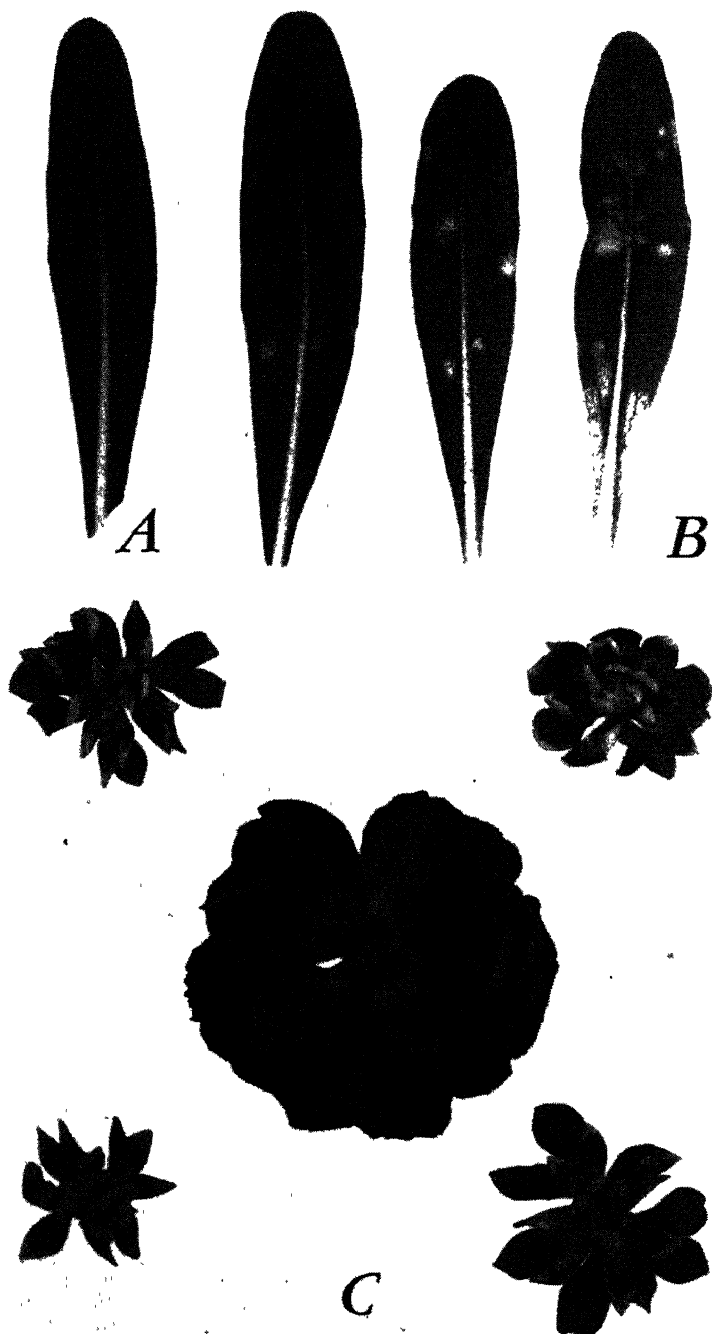


Plate 2. Symptoms produced by feeding of noninfective turnip aphid, *Rhopalosiphum pseudobrassicae*, on annual stock, *Mathiola incana* var. *annua*: A, leaf from healthy check or control plant on which no aphids had fed; B, pale-green circular areas around mouth-part punctures; C, center, normal flower of the Fiery Blood Red variety; grouped around it are four dwarfed flowers with tips of petals yellow or white or with all petals yellowish green.

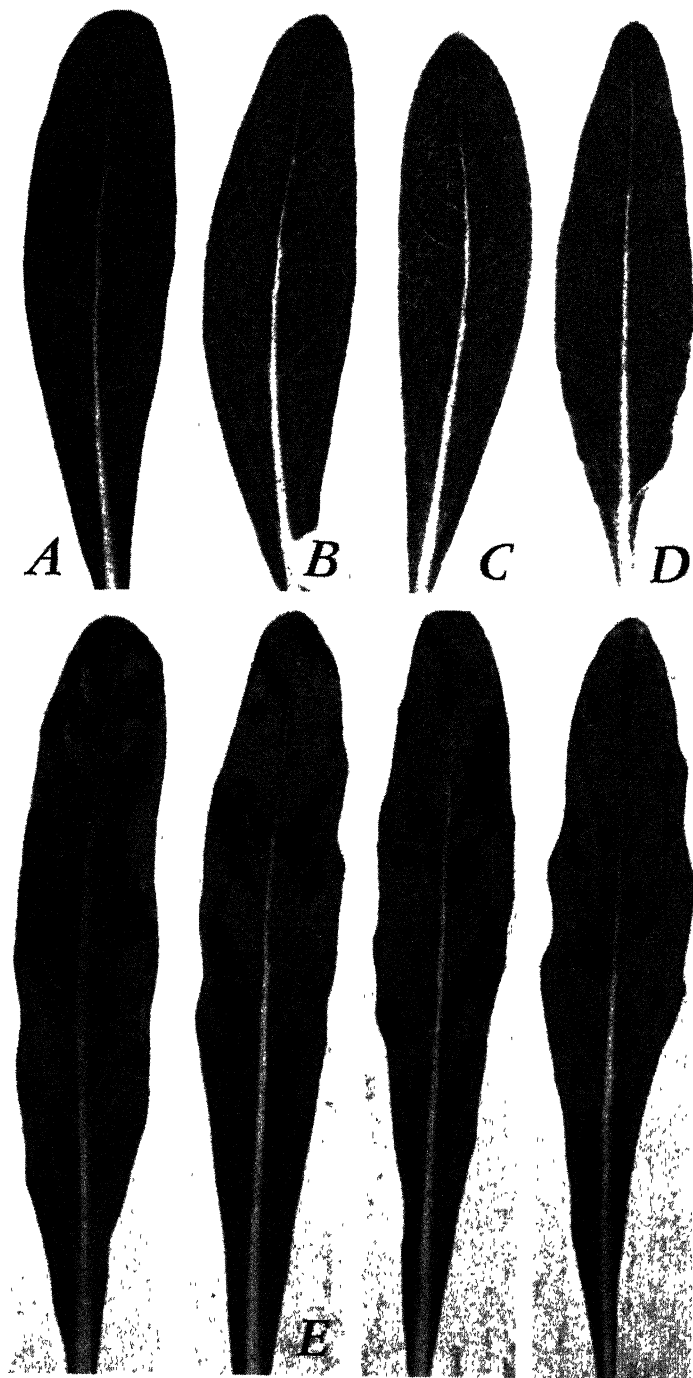


Plate 3. Symptoms produced by mild-mosaic virus on young leaves of annual stock, *Mathiola incana* var. *annua*: A, leaf from healthy plant; B, C, D, stages in clearing of veins and veinlets; E, mottling consisting of irregular-shaped pale and dark-green areas on young leaves (from a plant infected by means of the turnip aphid).

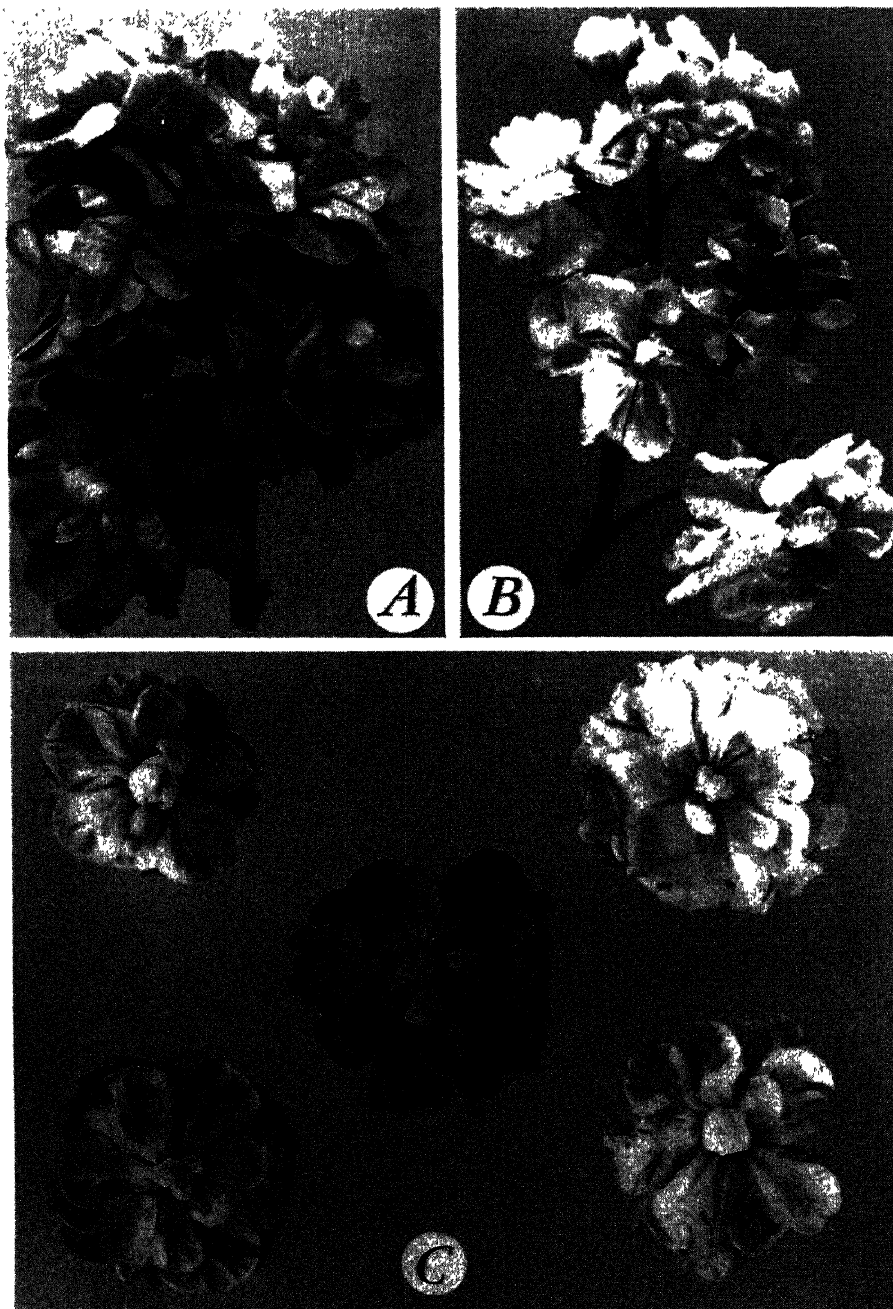


Plate 4. Field symptoms induced by mild-mosaic virus on flowers of naturally infected annual stock, *Mathiola incana* var. *annua*; Fiery Blood Red variety: *A*, apical cluster of flowers from a healthy plant; *B*, apical cluster of flowers showing breaking in color of petals from a naturally infected plant; *C*, center, normal flower; grouped around it are four flowers showing breaking, consisting of white areas and normal color. (Montara, San Mateo County, California, August 20, 1934.)

ADDITIONAL VIRUS DISEASES OF SPINACH IN CALIFORNIA¹

HENRY H. P. SEVERIN²

SUMMARY

Spinach was demonstrated to be naturally infected in California with sugar-beet mosaic and two cucumber mosaics—western cucumber mosaic and celery calico. Nine varieties of spinach were experimentally infected with the two cucumber mosaic viruses, five with sugar-beet mosaic. The three viruses were recovered from naturally infected spinach and transferred by mechanical inoculation to White Spine cucumber (*Cucumis sativus*), Turkish tobacco (*Nicotiana tabacum*), *N. glutinosa*, or sugar beet (*Beta vulgaris*).

The symptoms of these three diseases on spinach are similar. Filamentous leaves occur only in western cucumber mosaic. But cleared venation, chlorotic spotting, blisterlike elevations, and necrosis occur in all three. The viruses can be distinguished by transferring them to sugar beets; on that host the symptoms are sufficiently distinctive to permit identification.

New Zealand spinach (*Tetragonia expansa*) was experimentally infected with western cucumber mosaic, and the virus was recovered and transferred to White Spine cucumber and sugar beet.

The green peach aphid, *Myzus persicae* (Sulzer), is the most important vector of the western-cucumber-mosaic, celery-calico, and sugar-beet-mosaic viruses to spinach under natural conditions. The bean or dock aphid, *Aphis rumicis* Linnaeus, rarely transmits these viruses.

INTRODUCTION

FIVE VIRUS DISEASES have been reported to occur on spinach (*Spinacia oleracea*, family Chenopodiaceae) under natural conditions in California; namely, aster yellows (Severin, 1934; Severin and Frazier, 1945),³ sugar-beet curly top (Severin and Henderson, 1928; Scott, 1935), sugar-beet mosaic (Severin and Drake, 1948), spinach yellow dwarf (Severin and Little, 1947), and spotted wilt (Gardner, Tompkins, and Thomas, 1937).

This paper deals with two additional naturally occurring virus diseases of spinach—western cucumber mosaic and celery calico—and with sugar-beet mosaic on this host. Studies were made of the succession of symptoms on naturally and experimentally infected spinach caused by the three viruses. The symptoms of western cucumber mosaic were also studied on New Zealand spinach, *Tetragonia expansa*, which belongs to the family Aizoaceae. No insect-transmission tests are reported here. But previous reports on aphid transmission and on the aphid species naturally occurring on spinach are reviewed to determine which species are important vectors of the viruses.

¹ Received for publication May 11, 1948.

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³ See "Literature Cited" for citations, referred to in the text by author and date.

WESTERN CUCUMBER MOSAIC

Western cucumber mosaic occurs on several hosts in interior regions of California, but has not been found to occur naturally outside this region.

Outbreaks. During the spring of 1947, spinach in one 30-acre field near Patterson, in the northern San Joaquin Valley, was badly affected with yellowing of the foliage and rotting of the heart leaves. At first the injury was attributed to killing of the heart leaves by frosts. There was no evidence, however, of similar symptoms on spinach in other fields in the same district.

Later Bacon and Walz (1947) demonstrated that the rotting of the heart leaves resulted from the injuries of the seed-corn maggot, *Hylemya cilicrura* (Rondani), which also destroyed the young crown leaves of many plants.

Yellowing of the foliage, however, was present on many plants that showed no evidence of injury by the seed-corn maggot. On such plants the leaves showed large, blisterlike elevations (plate 1, *A, B*); some were malformed, thick, and leathery (plate 1, *B, C*). Since these symptoms suggested a virus disease, attempts were made to recover a virus. The western-cucumber-mosaic virus was recovered from spinach plants with yellow foliage, whether they showed injury from seed-corn maggot or not; it was transferred by the carborundum method (Rawlins and Tompkins, 1936) to sugar beets, which developed the typical symptoms (Severin and Freitag, 1948).

During the spring of 1948, a serious outbreak of western cucumber mosaic occurred on spinach in many fields near Westley and Patterson. The spinach turned yellow in one 40-acre field planted in September and was disked up in December. The 40 acres were replanted and again the spinach turned yellow and again was disked up. The virus was recovered from the second planting and transferred to sugar beets and squash. Spinach was demonstrated to be naturally infected with western cucumber mosaic in five other fields.

Symptoms. A noticeable symptom on naturally infected spinach, when the fields were viewed from a distance, was the yellow color of the entire plants, or yellow outer and green inner leaves; numerous dried or dead plants could be seen. A closer examination of some infected plants showed cleared veinlets on the youngest leaves (plate 2, *A*); and on intermediate leaves, circular chlorotic areas (plate 2, *C*), yellow blotches (plate 2, *B*), and necrotic areas in the yellow blotches (plate 2, *D*). Blisterlike elevations and malformations occurred on the heart leaves (plate 3, *A, B*). In the late stage of the disease, the youngest leaves are misshapen (plate 3, *D*, upper row) and frequently surround filamentous leaves (plate 3, *D*, lower row). When infected plants develop seedstalks, the filamentous leaves are very conspicuous (plate 3, *C*). In 1947 the blisterlike elevations were the prevailing symptom; in 1948 the filamentous leaves were prevalent and the blisterlike elevations rare.

The first symptom to appear on the youngest leaves of experimentally infected Bloomsdale (or Savoy-leafed) spinach is a clearing of the veins and veinlets (plate 4, *A*), accompanied with small, circular, chlorotic spots (plate 4, *A, B*). The younger leaves become malformed with blisterlike elevations (plate 4, *C, D*). Sometimes the younger leaves are folded along the midrib. In the late stage of the disease the newly developing leaves of some infected plants are linear, with blades reduced to but little more than the midrib.

Susceptible Varieties. The following nine varieties of spinach were experimentally infected with western cucumber mosaic: Bloomsdale or Savoy-leafed, Long Standing Bloomsdale, Broad Flanders, Giant Thick-leafed Nobel, Juliana, King of Denmark, Prickly Seeded, Virginia Savoy, and Viroflay.

The virus was recovered from each variety and transferred by mechanical inoculation to White Spine cucumber and to sugar beets.

New Zealand Spinach. The symptoms of western cucumber mosaic which appear on the leaves of New Zealand spinach, *Tetragonia expansa*, are wide yellow or pale-orange rings surrounding green tissue (plate 5, *A*). Later the green areas become chlorotic (plate 5, *B*) and often fuse (plate 5, *C*). The leaves frequently become malformed (plate 5, *D*). The yellow or orange rings may become necrotic. Necrosis of the margin of the leaf occurs. Later the entire leaf becomes dry.

The virus was recovered and transferred by mechanical inoculation to White Spine cucumber.

CELERY CALICO

Celery calico is a cucumber-mosaic virus which is common in the coastal fog belt and also occurs in the hot interior regions of California.

Natural Infection. The virus of celery calico was recovered from naturally infected spinach plants collected in vegetable fields near San Pablo. The virus extract was mechanically inoculated into healthy Long Standing Bloomsdale spinach, White Spine cucumber (*Cucumis sativus*), Turkish tobacco (*Nicotiana tabacum*), and *N. glutinosa*; typical symptoms of celery calico developed.

Symptoms. The older leaves of naturally infected spinach are lemon yellow (fig. 1, *A*). The younger leaves are bunched, narrowed, and deep green, with blisterlike elevations.

The first symptom to appear on the youngest leaves of inoculated varieties of spinach is a clearing of the veins and veinlets (plate 6, *A*) surrounding green areas (plate 6, *B*). The next symptom on the youngest leaves is the appearance of blisterlike elevations (plate 6, *C*); and the youngest developing leaves are narrowed and cupped downward. Later, necrosis of the younger leaves occurs. The oldest leaves become orange or lemon yellow (plate 6, *D*).

These symptoms are so similar to those of western cucumber mosaic (page 554) and sugar-beet mosaic (page 557) on spinach that a field infection of a spinach mosaic disease cannot be certainly identified by the symptoms on that host. It can be identified by transferring the virus to sugar beets; for on that host the symptoms are distinguishable (Severin, 1948).

Susceptible Varieties. The nine varieties of spinach infected with western cucumber mosaic were also experimentally infected with celery calico. The virus was recovered and transferred by mechanical inoculation to White Spine cucumbers, Turkish tobacco, and sugar beets.

SUGAR-BEET MOSAIC

Natural Infection. Spinach was demonstrated to be naturally infected with sugar-beet mosaic in vegetable gardens near San Pablo. The virus extract from diseased spinach collected in the field was inoculated in healthy spinach and sugar-beet plants, and typical symptoms of the disease developed.



Fig. 1. *A*, Symptoms of celery calico on a naturally infected plant of Long Standing Bloomsdale spinach, showing older leaves lemon yellow, and younger leaves bunched, narrowed, and deep green, with blisterlike elevations. *B*, Symptoms of sugar-beet mosaic on a plant of Long Standing Bloomsdale spinach, showing small chlorotic areas and cleared veinlets.

Symptoms. The symptoms on experimentally infected Giant Thick-leaved Nobel, Long Standing Bloomsdale, Prickly Seeded, Virginia Savoy, and Viroflay spinach are similar in most respects. The first symptom to appear is small, chlorotic areas on or between the veinlets and usually near the base of the youngest leaves (plate 7, *A*). These occur simultaneously with or are followed immediately by a broken type of cleared veinlets (fig. 1, *B*); in the greenhouse these cleared veinlets appear 10 days after inoculation. Numerous chlorotic rings develop, each with a necrotic center (plate 7, *B*). The young leaves assume a horizontal position (fig. 1, *B*). Later the chlorotic rings coalesce to form irregular chlorotic areas intermingled with conspicuous, dark-green, blisterlike elevations (plate 7, *C*), sometimes followed by chlorotic veinbanding (plate 7, *D*).

Contrary to Hoggan's (1933) observations, malformation on the youngest leaves is common on infected spinach here. Some dwarfed misshapen leaves show chlorotic areas and others blisterlike elevations (plate 8, *A, B*); others are twisted along the midribs (plate 8, *C*) or folded along the midribs (plate 8, *D*). Some of the youngest leaves are cupped outward, others are asymmetrical.

As the disease progresses, the older leaves show chlorotic rings which usually coalesce to form large, irregular, chlorotic areas interspersed with dark-green blotches (plate 8, *E*). Sometimes the chlorotic areas are smaller and more numerous, and the dark-green areas appear blisterlike, as on the younger leaves.

In the advanced stage of the disease, the older leaves usually show large, irregular, diffuse, yellowish areas which later become dark yellow or orange. Long Standing Bloomsdale and Virginia Savoy spinach developed numerous chlorotic rings, sometimes lenticular in shape, and measuring 4 mm in diameter, which later become necrotic. On all varieties the older leaves usually develop necrotic tissue at the tip; the necrosis gradually advances toward the base of the leaves; often it occurs along the margin or within the blade (plate 8, *F*). Necrosis gradually spreads over the older leaves and then toward the heart leaves. The dead tissue is papery, brown in color, and suggestive of sunburn. The plant finally dies.

Since spinach was so severely affected by the sugar-beet-mosaic virus, it may be of greater economic importance than is now realized.

Incubation Period. The incubation period of the disease in five varieties of spinach experimentally infected with sugar-beet mosaic ranged from 7 to 15 days. The number of times that the virus was recovered has been reported in a previous paper (Severin and Drake, 1948).

APHID VECTORS

Patch (1938) lists the following species of aphids as occurring on spinach under natural conditions.

- Cotton or melon aphid, *Aphis gossypii* Glover
- Bean or dock aphid, *Aphis rumicis* Linnaeus
- Hyalopterus atriplicis* Linnaeus
- Potato aphid, *Macrosiphum solanifolii* Ashmead
- Green peach aphid, *Myzus persicae* (Sulzer)

Bacon and Walz (1947), who carried on extensive tests on the aphid populations on spinach in the San Joaquin, Santa Clara, and Salinas valleys during the springs of 1946 and 1947, found that the green peach aphid, *Myzus persicae*, was the most abundant species on spinach. The bean or dock aphid, *Aphis rumicis*, was occasionally taken on spinach, and some of the females had given rise to small colonies of aphids. Winged forms of the pea aphid, *Macrosiphum pisi* (Kaltenbach), were observed on spinach but were not multiplying. Previous tests (Severin, 1942; Severin and Freitag, 1948; Severin and Drake, 1948) have shown that the green peach aphid transmits the western-cucumber-mosaic, sugar-beet, and celery-calico viruses; and that the bean aphid rarely transmits the first two. Hence, and also because of its abundance on spinach, the green peach aphid is the most important vector of these viruses to spinach, under natural conditions. The other species of aphids listed by Patch as occurring on spinach were not tested.

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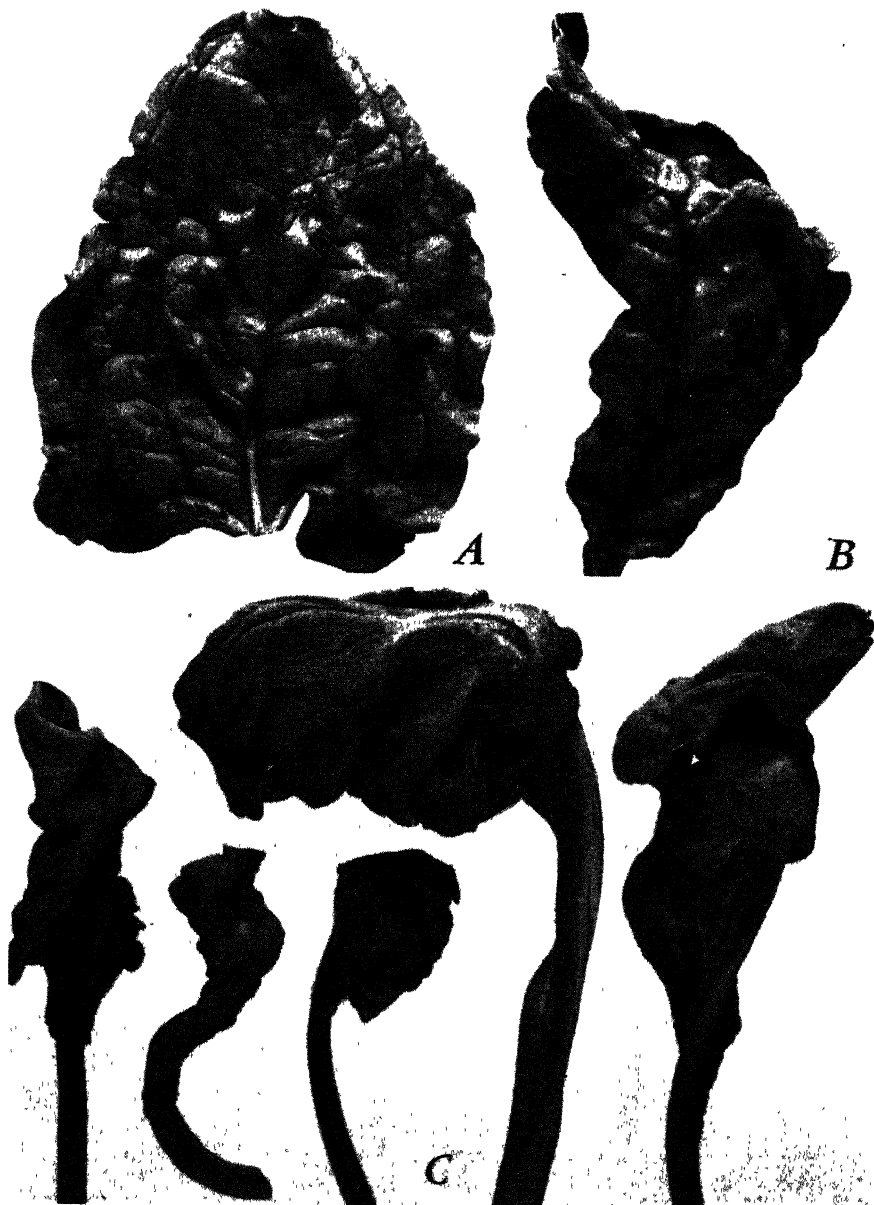


Plate I. Symptoms of western cucumber mosaic on leaves of naturally infected spinach, *Spinacia oleracea*: *A*, blisterlike elevations; *B*, malformed and blistered younger leaf; *C*, misshapen leaves.

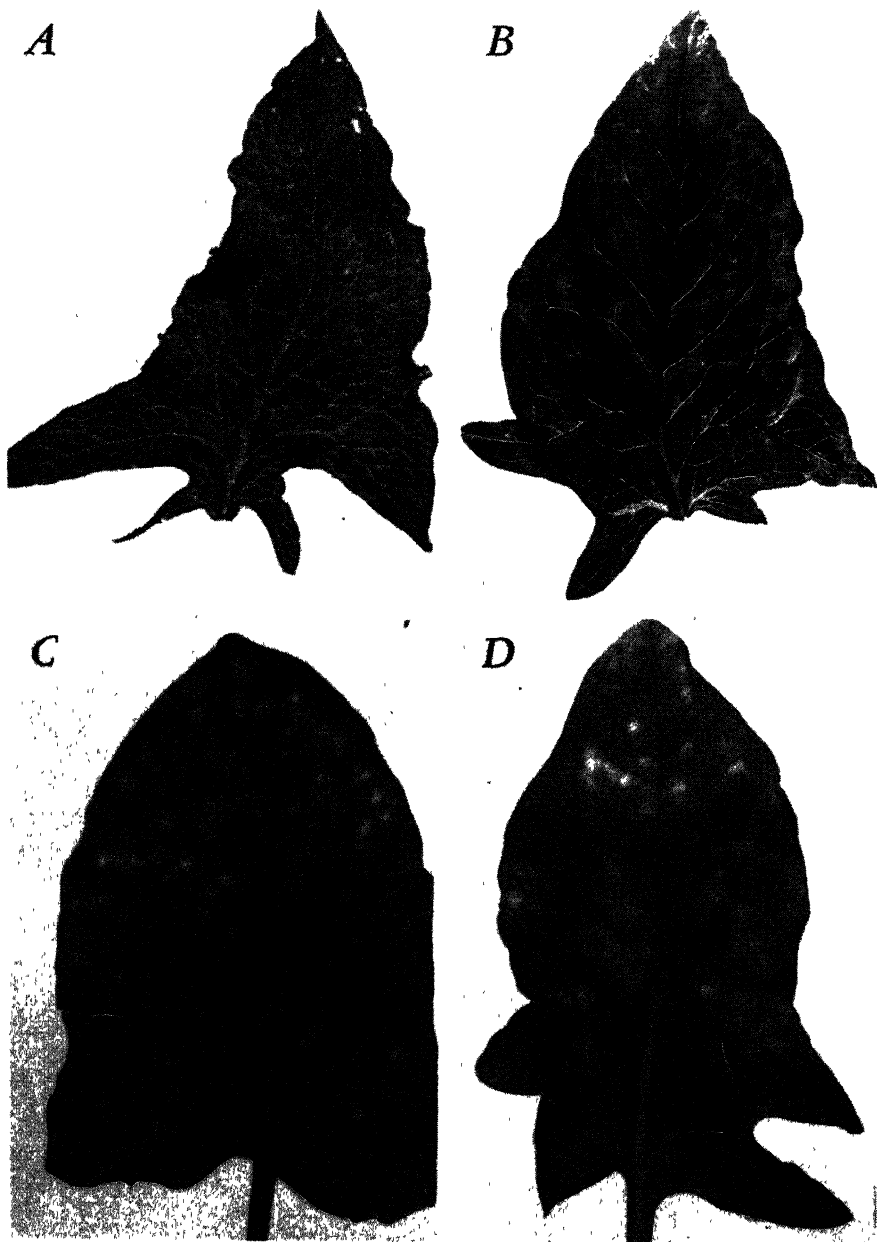


Plate 2. Symptoms of western cucumber mosaic on leaves of naturally infected spinach: *A*, cleared veinlets on youngest leaf; *B*, yellow blotches; *C*, circular chlorotic areas; *D*, necrotic areas in yellow blotches, on intermediate leaf. These symptoms and those shown in plate 1 cannot be distinguished with certainty from corresponding symptoms produced by celery calico and sugar-beet mosaic on spinach.

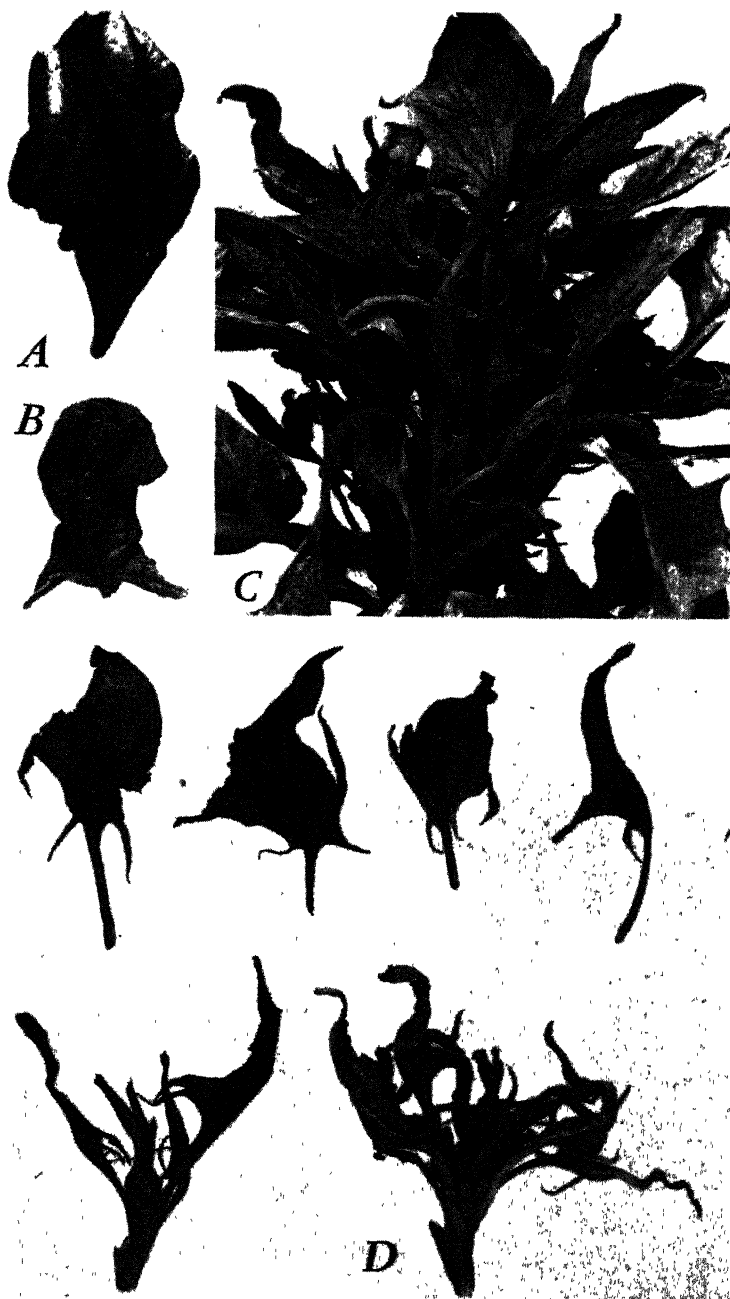


Plate 3. Symptoms of western cucumber mosaic on naturally infected spinach: *A, B*, blisterlike elevations and malformations on heart leaves; *C*, malformed and filamentous leaves growing from seedstalk; *D*, upper row, misshapen leaves; *D*, lower row, malformed leaves surrounding filamentous leaves.

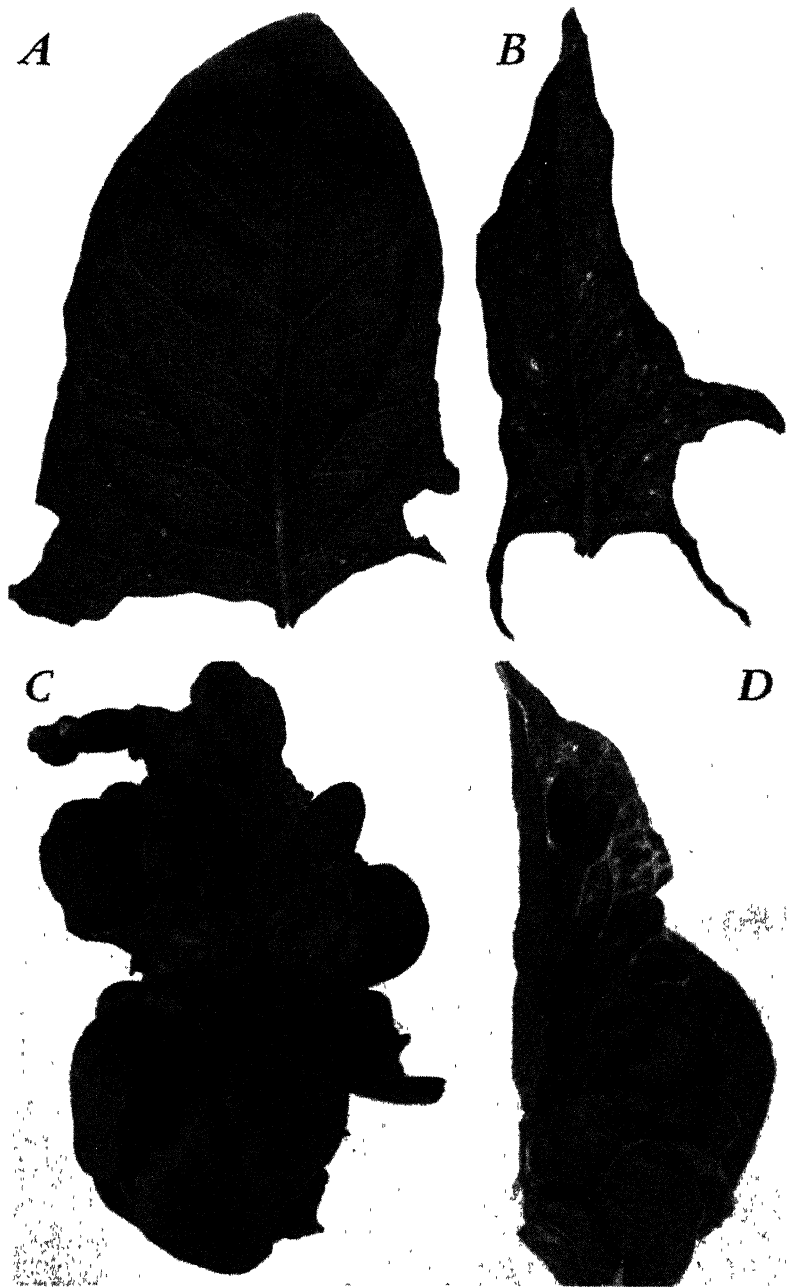


Plate 4. Symptoms of western cucumber mosaic on leaves of experimentally infected Bloomsdale spinach: *A*, cleared veins and veinlets accompanied by small, circular, chlorotic spots on youngest leaf; *B*, narrowed leaf showing numerous chlorotic spots, some fusing; *C*, *D*, malformed leaves with blisterlike elevations and apical chlorosis.

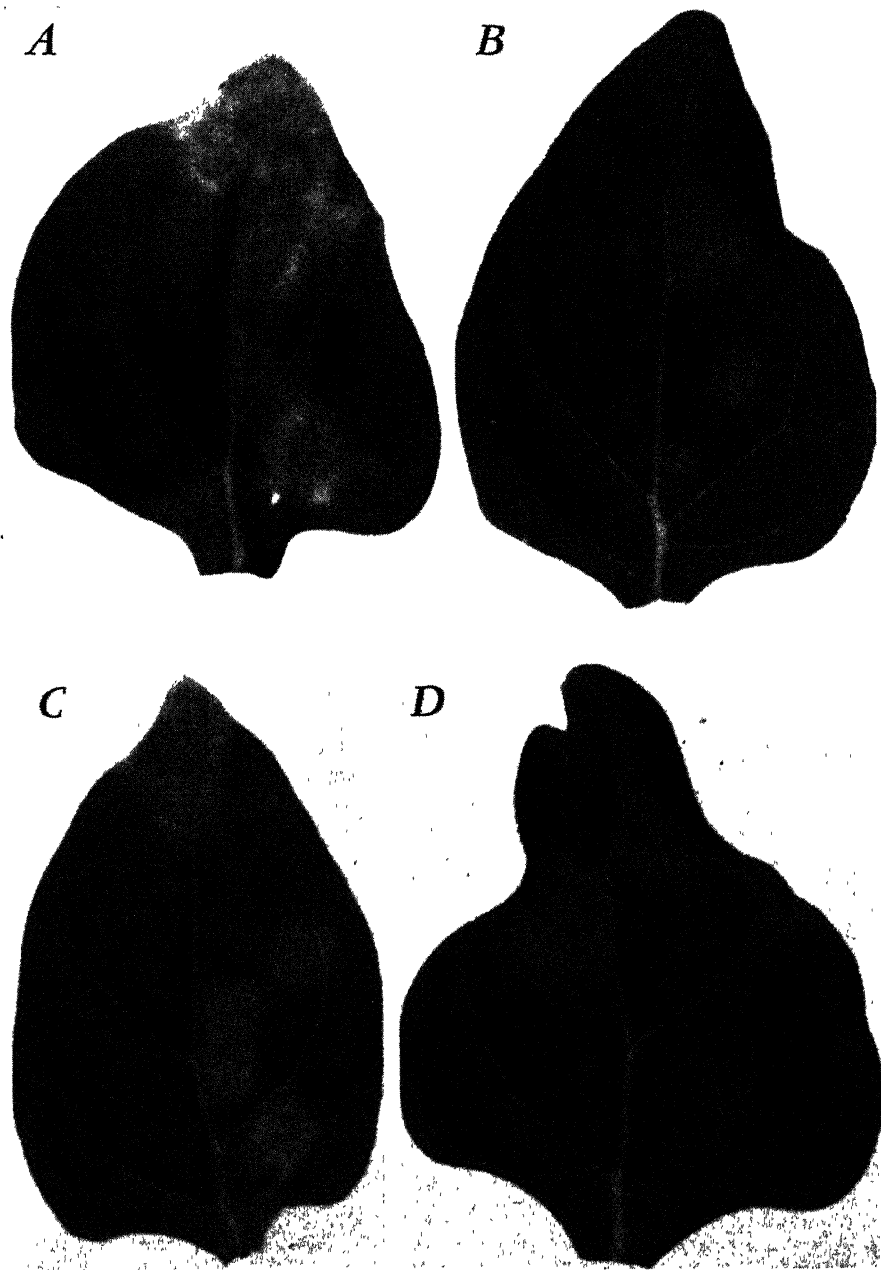


Plate 5. Symptoms of western cucumber mosaic on leaves of experimentally infected New Zealand spinach, *Tetragonia expansa* (family Aizoaceae): *A*, wide yellow or orange rings which surround green tissue; *B*, circular chlorotic areas; *C*, circular chlorotic areas fusing; *D*, malformed leaf with chlorotic areas. Blisterlike elevations and filamentous leaves, such as occur on spinach, have not been observed on this species.

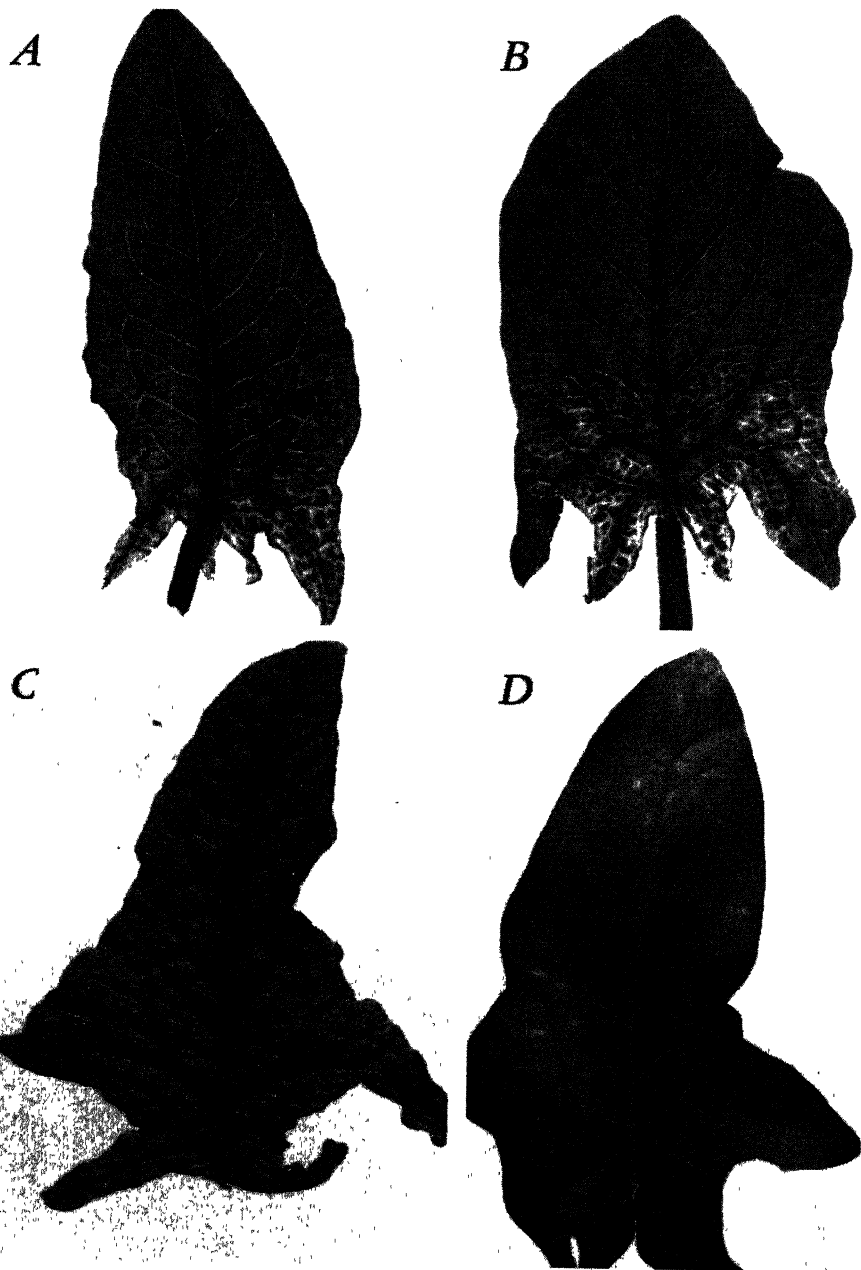


Plate 6. Symptoms of celery calico on leaves of experimentally infected Long Standing Bloomsdale spinach: *A, B*, youngest leaf, showing cleared veins and veinlets, surrounding green areas in *B*; *C*, blisterlike elevations; *D*, oldest leaf showing orange or lemon-yellow discoloration.

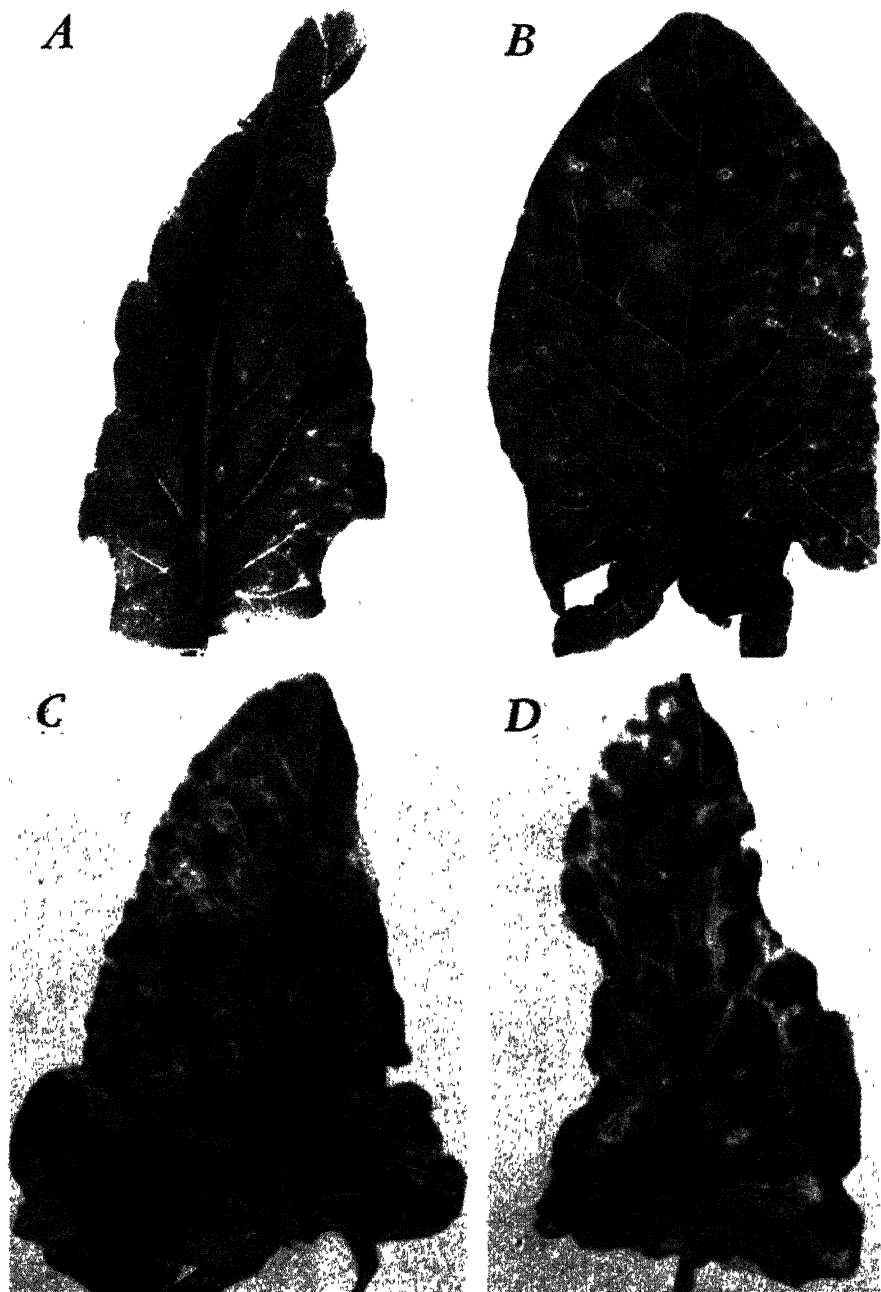


Plate 7. Symptoms of sugar-beet mosaic on young leaves of Long Standing Bloomsdale spinach: *A*, small chlorotic areas on and between the veins; *B*, small, circular, chlorotic rings, each with a minute necrotic center; *C*, chlorosis and blisterlike elevations; *D*, blisterlike elevations and veinbanding.

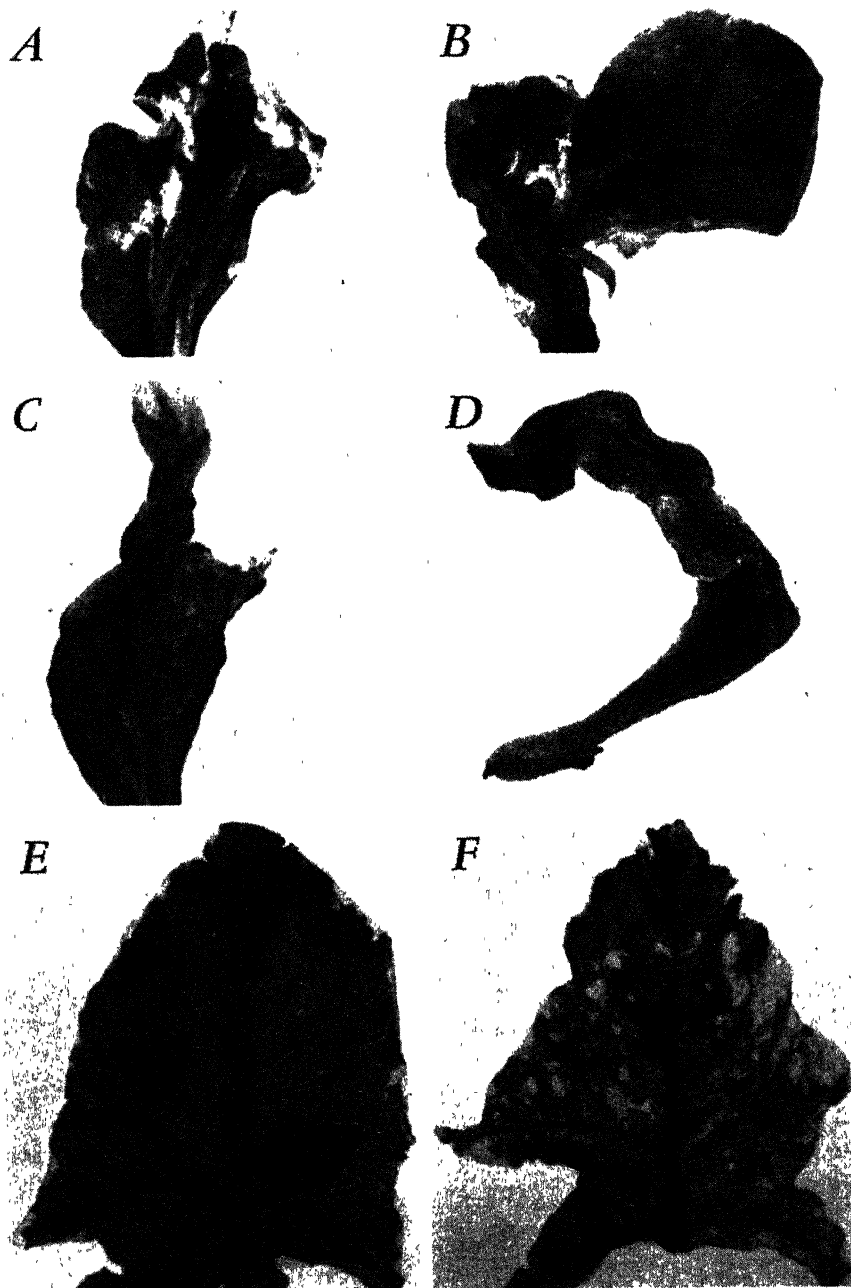


Plate 8. Symptoms of sugar-beet mosaic on leaves of Long Standing Bloomsdale spinach: *A, B*, dwarfed, malformed, young leaves showing blisterlike elevations; *C*, young leaf twisted along the midrib; *D*, young leaf folded along the midrib; *E*, old leaf showing large, irregular, chlorotic areas and dark-green blotches; *F*, old leaf showing necrosis along the margin and within the blade.

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BIOLOGY OF THE FIG SCALE IN CALIFORNIA¹

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INTRODUCTION

SINCE its introduction into California, the fig scale, *Lepidosaphes ficus* (Signoret), has become widespread in the fig-growing areas of the Central Valley. Although the scale has been tolerated, especially on dried figs, heavy infestations are now recognized as definitely injurious to the fruit, with a resulting financial loss to the grower. In the fig-canning industry, for instance, the need for clean fruit can be easily recognized. Knowledge of control measures was essential, and this was gathered largely from the experience of the growers. The program was handicapped, however, by lack of information on the life history of the pest. The present study, with most of the work done in the vicinity of Fresno, was made in answer to the demand for more exact information in California.

DISTRIBUTION

According to Ferris (1937),⁴ the fig scale was originally described in France from cultivated figs growing at Cannes. Newstead (1901) quoted a record of its presence in England on figs imported from France in 1875. It was reported in Italy by Berlese (1903), Leonardi (1920), Silvestri (1940), and Lupo (1942). Other references to the fig scale were made by Colvée (1881), Fernald (1903), MacGillivray (1921), and others. Umnov (1940) reported it to be a minor pest of figs in the Crimea, and Kuwana (1925) reported it on pears in Japan.

The fig scale is thought to have been imported in California in 1905 with fig cuttings from Algeria. The infestation started at Fresno and spread somewhat slowly at first. In 1917, Roullard reported that the infestation was confined within a radius of about $\frac{3}{4}$ mile, where some 500 trees were involved. In 1931, Simmons, Reed, and McGregor reported that the scale had spread to a point about 60 miles south-southeast of the original infestation. The prevailing winds blow in this direction.

Evidence was also presented to show that the fig scale could be spread to Calimyrna fig trees in caprification by the use of infested capriffs. Attention

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⁴ See "Literature Cited" at the end of this paper for complete data on citations, referred to in the text by author and date of publication.

was again directed to this method of spread by Kinsley in 1938. He revealed that in 1936 a survey of 36 square miles in Merced County showed fig scale infestation only in the area exposed to infested caprifigs in 1932. Two years later the original infestation had spread $\frac{3}{4}$ mile south and west, but very little to the north and east. At that time in Merced County some 495 acres of figs, including Mission, Adriatic, and Calimyrna—but not Kadota—varieties were infested with fig scale.

Since 1938, the fig scale has attacked all varieties of figs in Merced County, and has spread north to Stanislaus County and to Stockton in San Joaquin County. However, the agricultural commissioners of the counties bordering

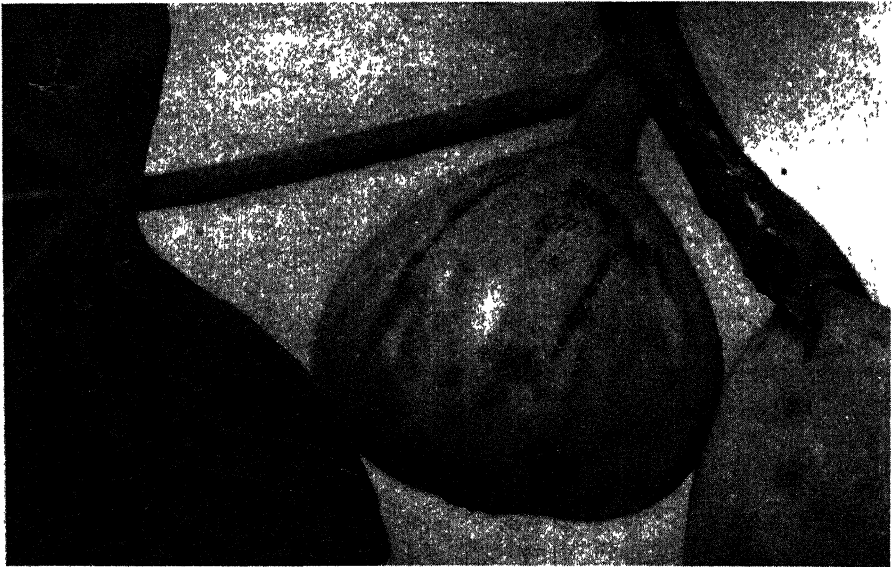


Fig. 1.—Fig scale on leaf and ripening fruit of Adriatic fig.

San Joaquin County on the west and north—Alameda, Contra Costa, Sacramento, Solano, and Yolo—have no records of the fig scale in their areas. In 1946, the writers surveyed two Kadota fig orchards in Brentwood, Contra Costa County, but found no scale. The State Bureau of Entomology and Plant Quarantine has specimens from San Jose, Santa Clara County.

What is believed to be an isolated infestation has existed in Glenn County for several years. A survey made in October, 1946, showed heavy infestations close to the city of Orland. Very light infestations were found at distances of 1 mile north, 3 miles west, about $2\frac{1}{2}$ miles east, and 2 miles south of Orland. At greater distances from Orland, but within Glenn County, scale was not found. The nearest known infestation from Orland is some 130 miles distant.

The most southerly general fig scale infestation in California is in Tulare County, where the degree of infestation varies from heavy near Dinuba and Orosi to light at the southern boundary. The State Bureau of Entomology and Plant Quarantine has several records of the occurrence of fig scale in both Kings and Kern counties.

NATURE AND IMPORTANCE OF INJURY

Fig scales on the leaves (fig. 1) often cause the area just beneath the scale to become chlorotic. Heavily infested leaves generally appear to be a lighter green than scale-free leaves. The greatest number of scales found on a single leaf was 1,042. Many growers believe that the scale causes the leaves to drop prematurely. The evidence supporting this belief is meager, however, as no study of such reaction has been made. On the twigs, fig scale populations may become so great as to almost encrust the newest growth. The effect of fig scale on the tree's vitality and its ability to set a crop has not been measured.

When light-colored varieties of fruit infested with fig scale approach maturity, the part beneath and immediately surrounding the scale remains dark green as the rest of the fig turns light green and yellowish (fig. 1). On Mission figs a red spot, lighter than the dark skin, appears beneath the scale. This spot remains light and conspicuous on the dried fruit. As the fruit becomes fully mature and begins to shrivel, the area beneath the scale loses its dark color and very often remains firm. This makes the dried fruit look warty. In contrast to normal dried Adriatic figs (fig. 2 A) the infested fruits are small, shriveled, spotted, and light in weight (fig. 2 B).

The dark-green spots which form beneath the scale during the ripening of canning figs will not cook out in processing. Fruit with more than three such marks is culled and put into jam stock, with a consequent loss of value. In 1944, when canning figs brought \$125 per ton and jam-stock figs \$85 per ton, this loss amounted to \$40 per ton. Since at present no regulations within the dried-fig industry require scale-free fruit, the amount saved financially by control of fig scale on drying figs is difficult to estimate. Nevertheless, the packers are becoming less interested in buying infested fruit. As a consequence, growers are increasingly recognizing the need for fig scale control.

A survey was made by the University of California in 1942 to determine the amount of spray material needed by fig growers for the control of fig scale. The survey disclosed that approximately 87,000 gallons of dormant oil were used on an estimated 20 per cent of the California fig acreage. Since 1942, dormant oil spraying for control of fig scale has become more or less general practice in Fresno and nearby counties. This would seem to indicate that the annual amount of control work has been greatly increased.

LIFE HISTORY AND FIELD BIOLOGY

Earlier observers (Roullard, 1917; Simmons *et al.*, 1931) who worked with the fig scale in the field assumed that there was only one species involved. Roullard, however, in 1917 observed that the scale on fruit and leaves was so different from that on twigs as to suggest the occurrence of more than one species. In a recent conversation, Mr. Roullard related how R. L. Nongaret, then with the United States Department of Agriculture, Bureau of Entomology, had started experiments to settle this question but had left before the work was completed. Ferris, on the basis of habitats and morphology of the adult females, decided that two species were involved, which he designated as *Lepidosaphes ficus* (Signoret), a twig-infesting form, and *Lepidosaphes fefoliae* (Berlese) primarily a leaf-infesting form.

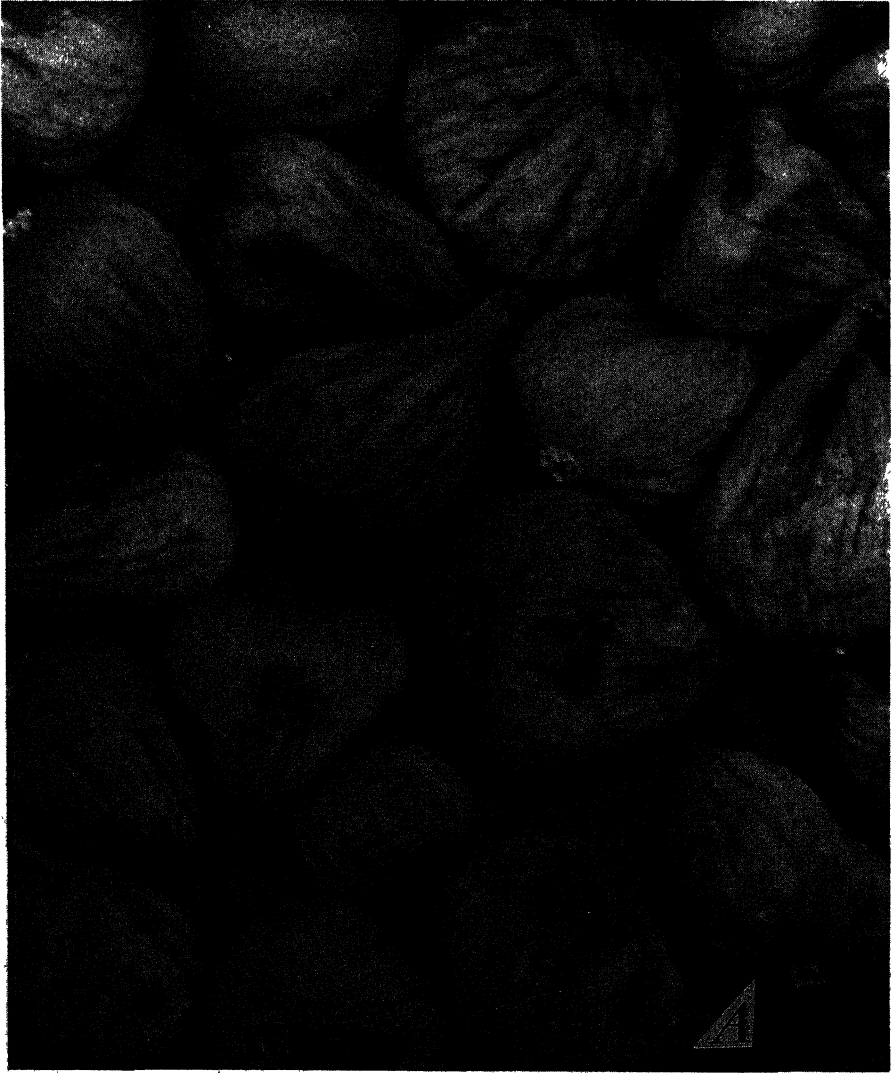


Fig. 2 A.—Dried Adriatic figs: Clean, uninfested fruit.

In Italy in 1943, however, Lupo showed that *L. ficifoliae* was a summer form of *L. ficus*. Although Lupo's work was published in 1943, war prevented his results from being brought to the attention of American workers until 1945. The work in this paper includes a confirmation of Lupo's conclusions.

Description. Descriptions and illustrations of the female bodies are given by Ferris (1937; 1938). The fig scale is a typical armored scale. It has a thick protective shell or scale above, and a thinner scale beneath the body.

In the winter, the female scales on the wood are dark brown with a thin waxy coating. The shape is much like that of a miniature oyster (fig. 3). At

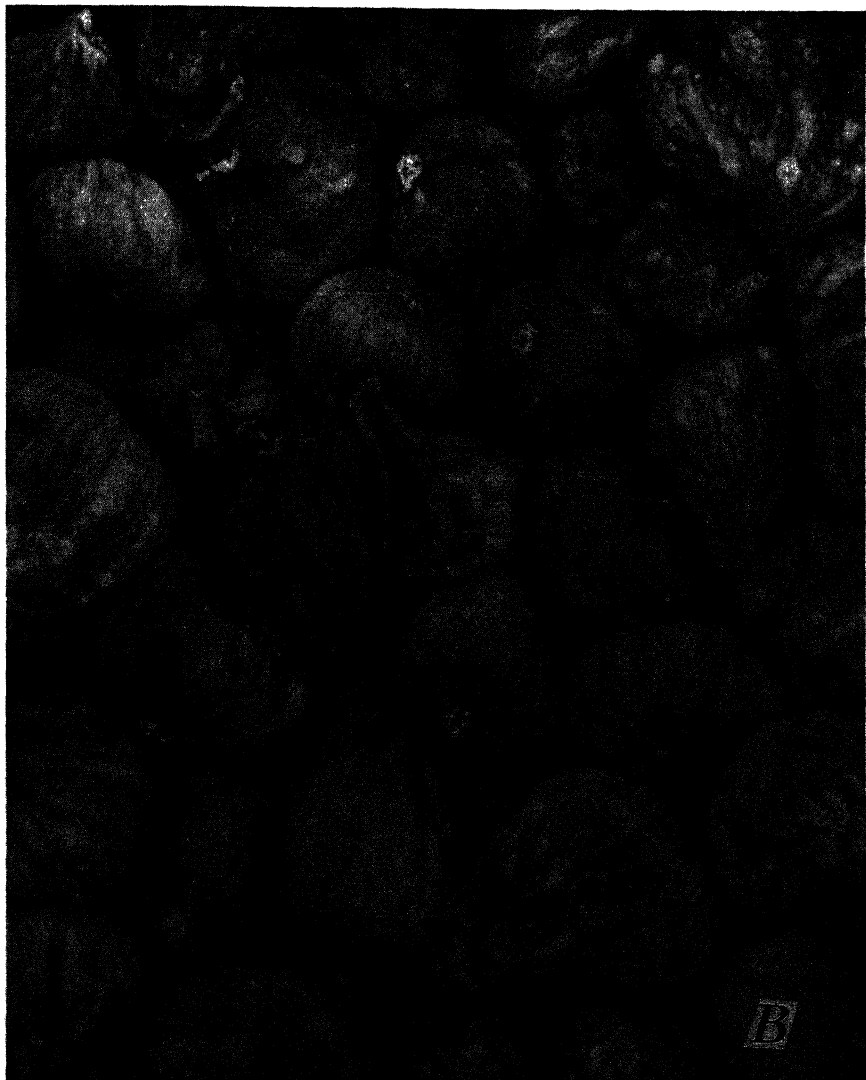


Fig. 2 B.—Dried Adriatic figs: Scale-infested fruit.

the narrower end of the female scale, the exuviae of the first and second stages of development may be seen. In the summer, the female scales on the leaves are much lighter and smaller than the winter scales. On the under surfaces of the leaves, the hairs and prominent veins often cause the scales to be laid down in distorted shapes. The scales on the top of the leaves are larger and darker colored than those on the lower surfaces, which are often whitish.

The males appear chiefly on the leaves. The scales of those on the upper surface are darker colored than those on the under surface. Only one exuvia appears in the scale of the male.

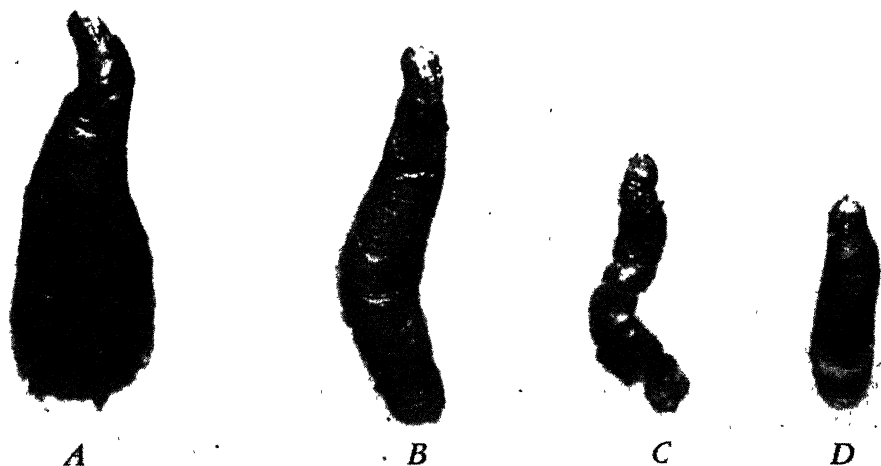


Fig. 3.—Shells of the fig scale: *A*, Overwintering female taken from a twig; *B*, Female from upper leaf surface; *C*, Female from lower leaf surface; *D*, Male from lower leaf surface. (Enlarged about 24 times.)

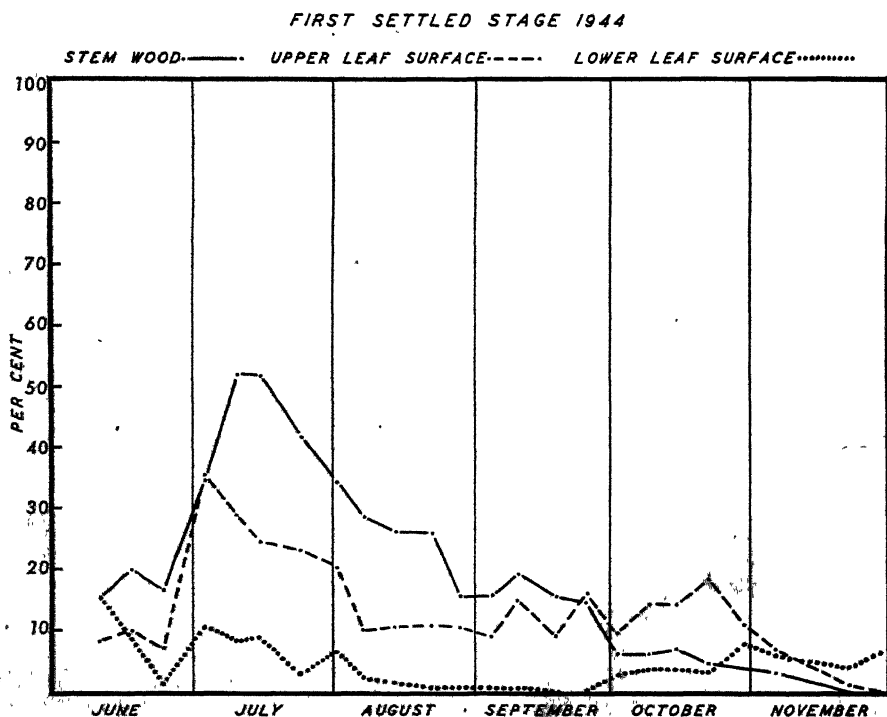


Fig. 4.—Relative abundance of the first settled stage fig scale at different locations on the tree in 1944.

Table 1 gives the length and width of scales selected at random.

TABLE 1
MEASUREMENT OF FIG SCALE SHELLS SELECTED AT RANDOM

Sex	Location on tree	Number measured	Average		Range	
			Length (mm)	Width (mm)	Length (mm)	Width (mm)
Female	Upper leaf surface	110	1.6	0.3	1.1-1.9	0.2-0.5
Female	Lower leaf surface	121	1.2	0.3	0.8-1.5	0.2-0.3
Female	Wood (in winter)	89	1.9	0.6	1.1-2.5	0.4-0.6
Male	Upper leaf surface	110	1.0	0.3	0.7-1.1	0.2-0.3

Location of Overwintering Females. In California early in 1944 observations were begun on the life history and field biology of the fig scale. First observed was the location of live and dead scales on various parts of dormant trees. Since the pruning and cultural practices for drying figs differ from the practices required for canning figs, and therefore produce a different type of new wood growth, both groups were included in the survey. Examinations were made on trees in Calimyrna, Adriatic, and Mission orchards which produced dried figs, and on trees in three Kadota orchards, one of which produced dried figs and the other two canning figs.

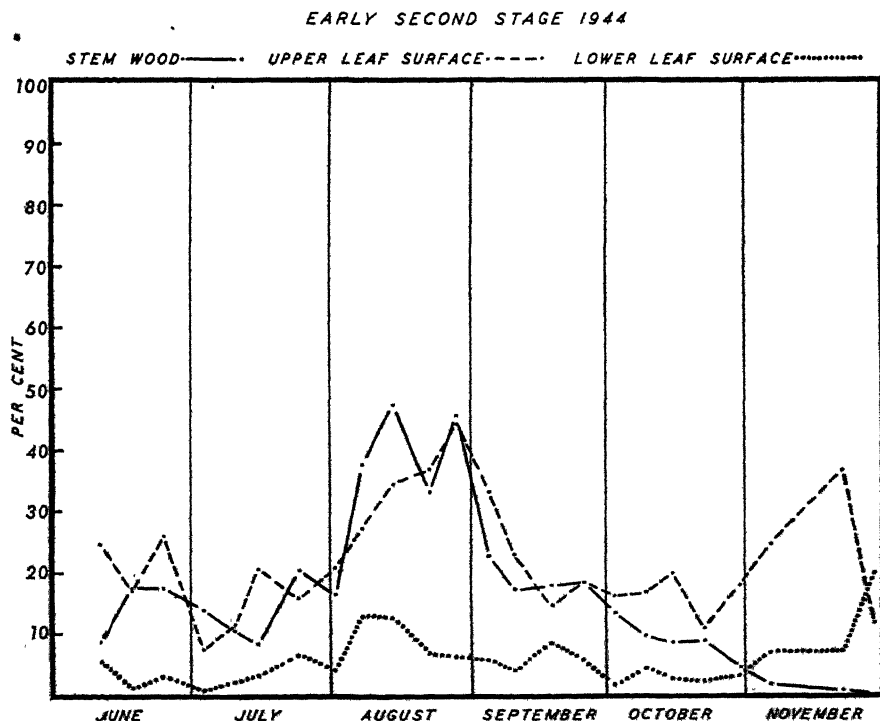


Fig. 5.—Relative abundance of early second stage fig scale at different locations on the tree in 1944.

TABLE 2
NUMBER OF LIVE FIG SCALE PER SQUARE INCH, AND PER CENT ALIVE ON LIMB WOOD OF DIFFERENT AGES IN JANUARY, 1944

Use	Variety	Number of live scale per square inch on wood of age indicated				Number of live scale as per cent of total scale examined on wood of age indicated				Number of live scale as per cent of total live scale on wood of age indicated				Total number of live scale examined
		One year old	Two years old	Three years old	Four years old or older	One year old	Two years old	Three years old	Four years old or older	One year old	Two years old	Three years old	Four years old or older	
Drying	Calimyrna	28 1	20.1	30.2	0.6	45.9	18.8	18.5	6.6	28.8	23.1	39.1	9.0	156
	Adriatic	23 8	21.7	3.5	2.2	85.9	60.3	34.9	47.6	65.6	29.0	3.3	2.0	4,511
	Mission	23 1	9.2	4.6	0.3	83.7	77.2	45.0	32.2	56.6	25.5	16.2	1.7	1,129
	Kadota	86.0	66.1	11.6	0.5	65.7	67.4	53.9	43.8	44.2	37.6	10.1	8.1	1,177
Canning	Kadota*	4 2	25.1	9.8	6.0	81.7	65.9	75.8	38.5	28.0	34.7	21.5	15.8	4,368
	Kadota†	27.7	41.4	14.4	3.2	92.7	88.9	53.4	39.7	40.6	40.3	4.2	14.9	1,504

* Sprayed 9 months to one year before examinations.

† Sprayed 21 months to two years before examinations.

Bark flakes from young shoots and from spots of heavy infestation on the trunks and main structural limbs were examined. The areas examined were 1,362 square inches from the limbs and 34 square inches from the trunks. Of the 20,702 scales examined—19,374 from limb wood and 1,328 from bark flakes—only 66 per cent contained living females. The scales on the youngest wood (1943) contained the largest per cent (82.5) of live females while the scales on the trunk contained only 12.3 per cent of live females. The same

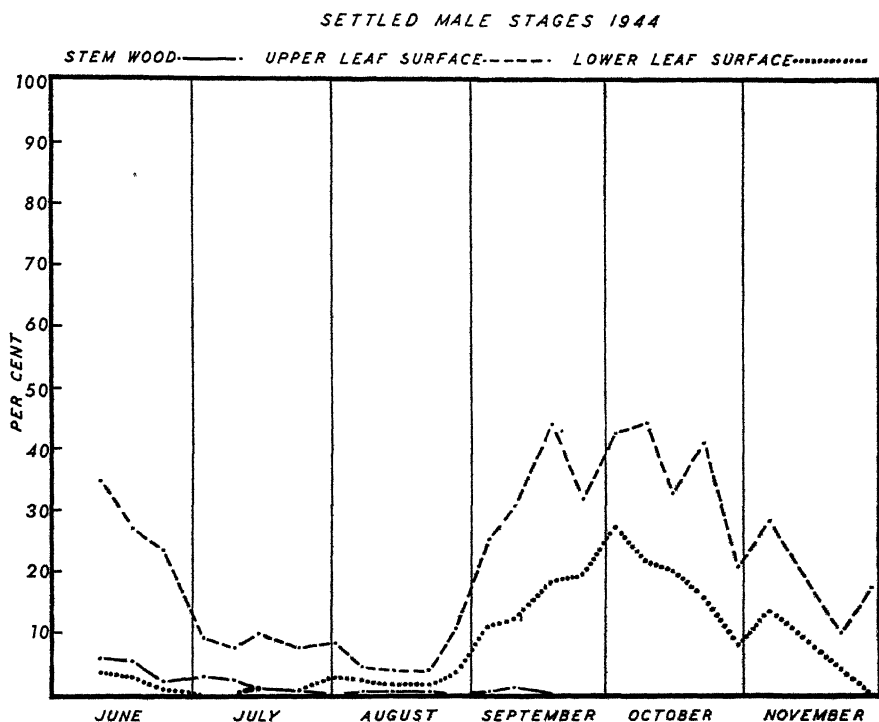


Fig. 6.—Relative abundance of settled male stages of fig scale at different locations on the tree in 1944.

observations also furnished data on the proportions and density of scale population on different parts of the tree. The observations are summarized in table 2.

The examination in January, 1944, of limb wood of different ages showed that on drying figs, about 60 per cent of the total live scale from wood of all ages was found on the youngest wood (produced during the 1943 growing season) and nearly 30 per cent on the wood produced in 1942. Similarly, on canning figs, 31 per cent of the live scale was on the wood produced in 1943 and 36 per cent on wood produced in 1942. Thus, on drying figs in the winter, one may expect to find about 90 per cent of all live scale on the wood produced during the past two growing seasons. On canning figs, however, one may expect to find only about 67 per cent of the total live scale on such wood. The live

scales on the trunk, which were restricted to the smoother and more succulent bark, made but a small per cent of the total. On the most heavily infested parts of the trunks of Calimyrna, Adriatic, and Mission varieties there were about 3 live females per square inch and on the Kadota variety about 9 per square inch.

The influence of cultural practices on the location of scale is evident when the number per square inch on the wood of the various years is examined.

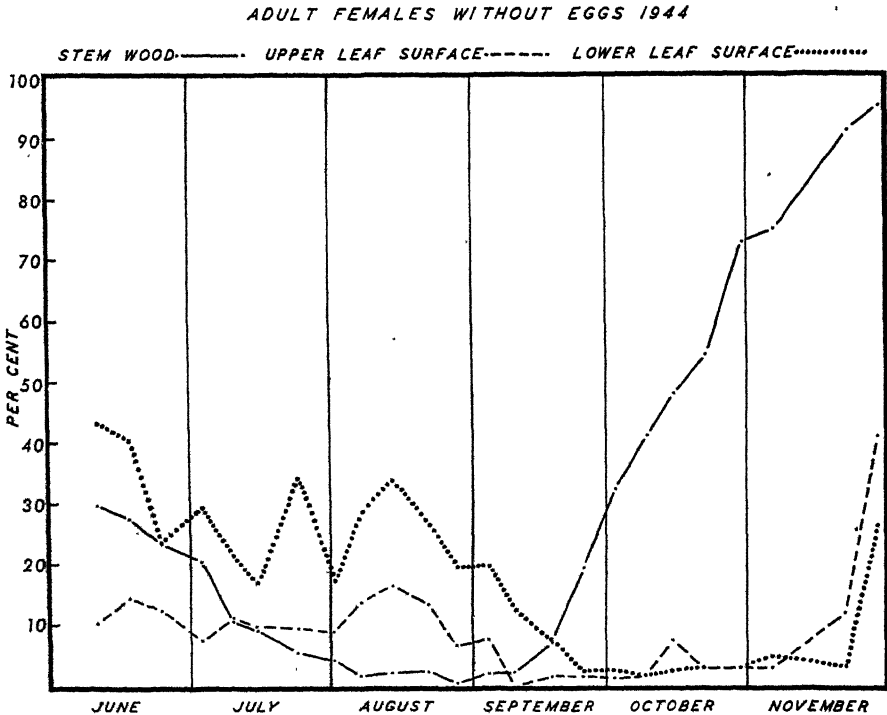


Fig. 7.—Relative abundance of adult female fig scale without eggs at different locations on the tree in 1944.

On trees which produce canning figs, most of the new wood is removed, and only a few buds are left. As a consequence, a substantial part of the infestation is removed in the pruning process. Heavy fertilization should accompany heavy pruning to induce vigorous shoot growth the following season. As a result of the removal by pruning of perhaps 40 per cent of the scale on the current season's wood, and the presentation of a large area of new wood surface to the progeny of this reduced population in the following season, the number of scales per square inch on new wood is smaller on canning than on drying figs. The greatest density occurs on the two-year-old wood.

Vigorous annual growth is not encouraged on trees producing drying figs, and little of the new wood is removed by pruning. As a result, a maximum number of scales remains on the trees, with heavy infestation on the limited amount of new wood.

The infestation figures presented indicate that live overwintering scales may be found at any point on the tree. In this study, even though the scales were heaviest on the newer wood, and decreased as the age of the wood increased, live scales could be found wherever succulent bark occurred above the ground. The necessity of covering the entire tree with dormant oil sprays in order to secure control was therefore indicated.

TABLE 3
OVIPOSITION OF OVERWINTERED FIG SCALE

Date	Locality	Scale from one- and two-year-old wood		Scale from trunk and main structural limbs	
		Number examined	Per cent with eggs	Number examined	Per cent with eggs
Observations, 1944					
February 7 . . .	Reedley	1,504	0.1
9.. . . .	Fresno.. . . .	1,828	0.0
10. . . .	Merced.	219	25.1
15 . . .	Fresno.	1,735	0.2
17.	Fresno.	1,216	0.5	63	25.4
23. . . .	Fresno.	1,202	0.1	293	14.3
28*. . .	Fresno.	1,326	5.1	286	51.1
March 2	Fresno	908	13.9
7†	Fresno	476	25.9	286	54.9
10. . . .	Fresno	490	55.9
13‡	Fresno.	878	85.4	245	90.6
16. . . .	Fresno.	1,686	88.0
20.	Fresno.	1,036	97.3
27§	Fresno.	379	99.7
Observations, 1945					
February 20*	Fresno.	300	4.7	71	46.5
26†	Fresno.	650	21.9	84	50.0
March 5.	Fresno.	400	61.5	226	86.3
12‡	Fresno	300	92.7	48	93.8

* Almonds in general bloom.

† Apricots beginning to bloom.

‡ Apricot bloom falling. Buds $\frac{1}{4}$ to 1 inch long on side of Adriatic fig trees.

§ Peaches generally in bloom. The more advanced Adriatic fig foliage $1\frac{1}{4}$ to 2 inches long.

Eggs of Overwintered Scale. In an attempt to associate the egg-laying period of the overwintered female scales with phenologic events in the vicinity of Fresno, certain observations were carried on in 1944 and 1945. Separate records were made for scales from one- and two-year-old wood and for scales from the trunks and main structural limbs. The observations are summarized in table 3.

In February of both 1944 and 1945, when almonds of the region were in bloom, about 5 per cent of the scales on the twig wood were ovipositing. When apricots were in bloom, the greatest increase in the per cent of female egg-laying scales was noted. In both years, about 20 per cent of the females had

ADULT FEMALES WITH EGGS HATCHING 1944

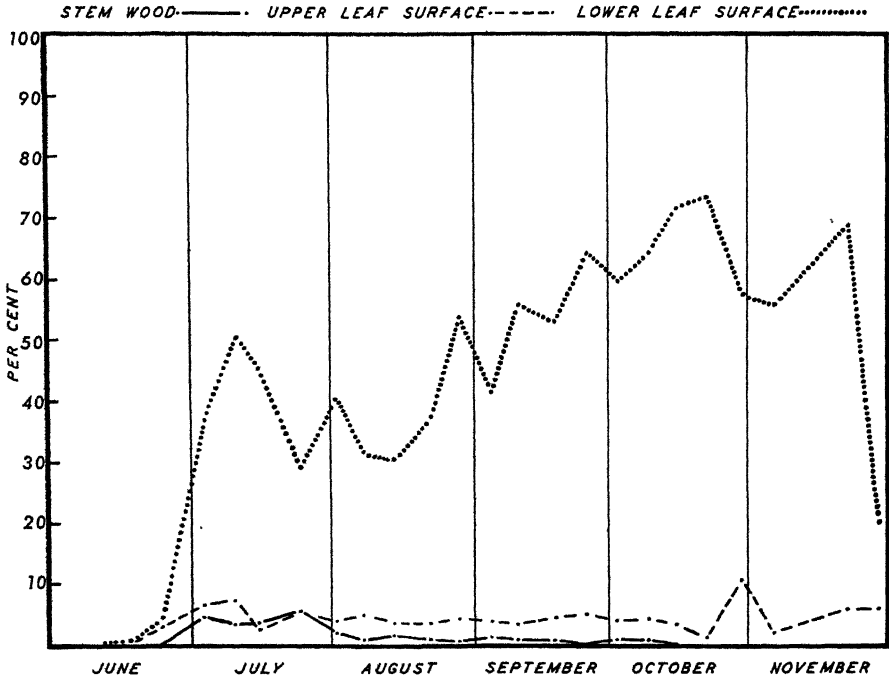


Fig. 8.—Relative abundance of adult female fig scales with eggs hatching at different locations on the tree in 1944.

FIRST SETTLED STAGE 1945

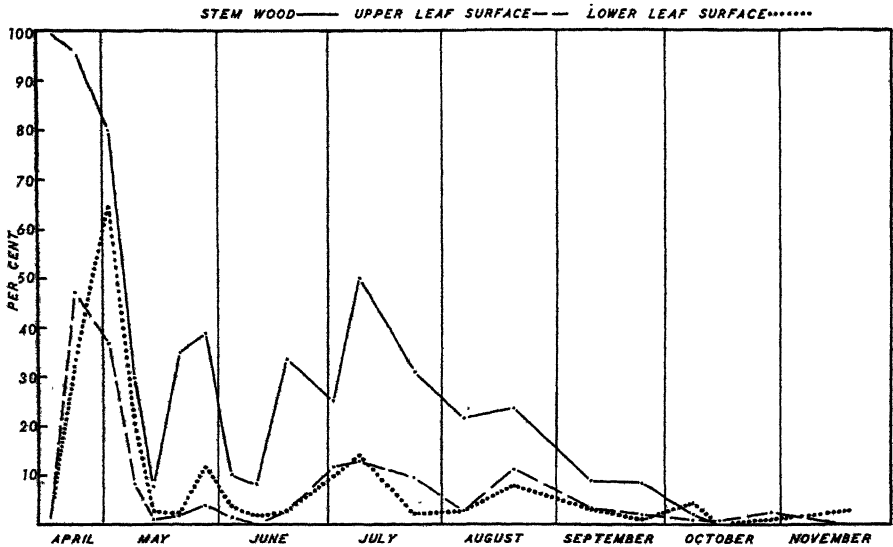


Fig. 9.—Relative abundance of first settled stage fig scale at different locations on the tree in 1945.

begun to oviposit when apricots began to bloom, and about 90 per cent were laying eggs when the bloom was falling. It was also noted that at this time the buds on the sides of Adriatic fig trees were about $\frac{3}{4}$ inch long.

The tendency for scales on the large wood to begin oviposition earlier than those on the new growth was noted in 1944 and in 1945. By mid-March, how-

TABLE 4
NUMBER OF EGGS FOUND UNDER OVERWINTERED FIG SCALE
ON ADRIATIC FIG TREES, 1944

Date	Number		Average number of eggs per scale
	Scales examined	Eggs found	
April 5.....	100	3,105	31.1
April 13.....	100	2,864	28.6
April 20.....	100	3,083	30.8
Total.	300	9,052	30.2

TABLE 5
HATCHING OF FIRST SUMMER BROOD FROM EGGS OF
OVERWINTERED SCALE, 1944

Date	Number		Per cent of eggs hatched	Per cent of scale with hatching complete
	Scales examined	Eggs observed		
April 5.....	100	3,105	4.4	0.0
13.....	100	2,864	14.1	0.0
20.....	100	3,083	20.9	0.0
26.....	45	1,522	37.5	0.0
May 5.....	50	1,681	58.4	0.0
11.....	14	514	63.2	0.0
19.....	15	470	59.4	13.3
23.....	20	828	72.8	10.0
June 5.....	17	670	75.4	17.7
12.....	20	629	87.6	50.0
24.....	10	269	96.7	90.0

ever, this difference was no longer apparent. It will be remembered, too, that the scales on the older wood form but a small part of the total overwintering population.

Overwintering female scales were selected at random—except for those attacked by predators—and were examined in April, 1944, for number of eggs. The results are summarized in table 4. The number of eggs per scale ranged from 0 to 51 with an average of 30.2. Simmons *et al.* (1931), give 10.9 eggs as the average number laid per female. The brood observed, however, is not stated.

The hatching period of the first summer brood from eggs laid by overwintered females was longer than 11 weeks. In 1944, as shown in table 5, hatching

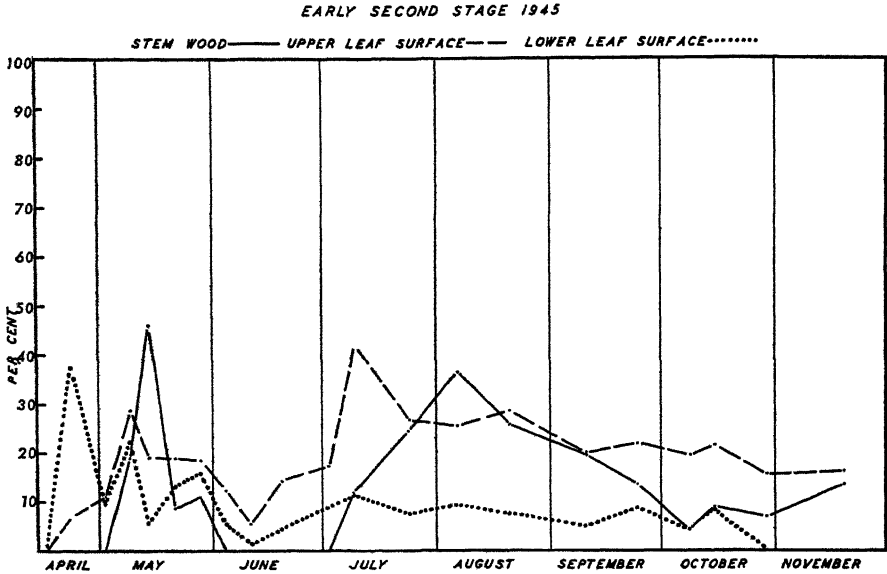


Fig. 10.—Relative abundance of early second stage fig scale at different locations on the tree in 1945.

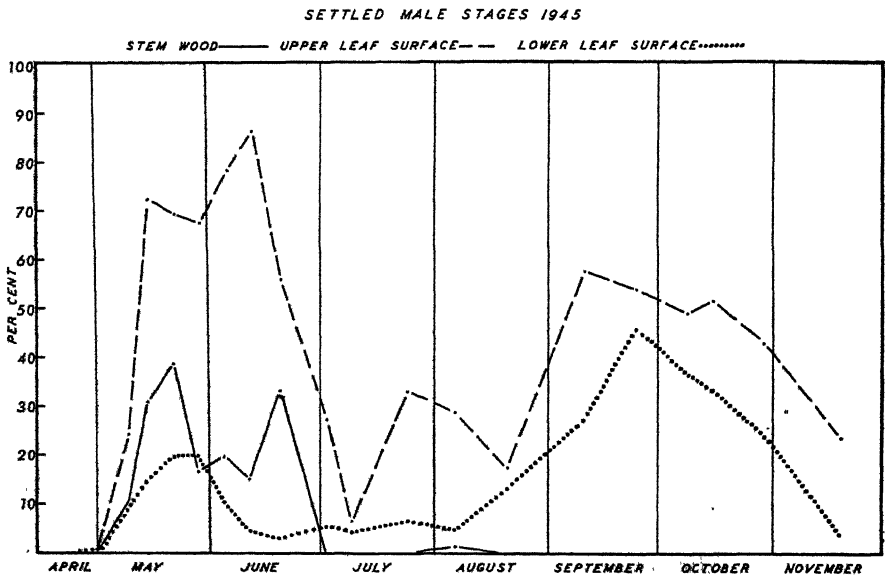


Fig. 11.—Relative abundance of settled male stages of fig scale at different locations on the tree in 1945.

had begun by April 5 and was not quite complete on June 24. The first complete hatch under a single scale was noted on May 19.

On June 24, eggs hatched from the first summer brood and the resultant second-brood crawlers were present on the leaves while the hatching of eggs from overwintered females was still incomplete. How long this overlapping had been going on is not known, but hatched eggs which had been laid by the first summer brood were first found on June 17. This fact had been previously observed by C. K. Fisher of the Bureau of Entomology and Plant Quarantine.

These observations, together with those on oviposition, showed that in 1944 the oviposition period of the overwintered females extended from February 7 to June 24.

TABLE 6
NUMBER OF EGGS FOUND UNDER FIRST SUMMER BROOD SCALE
ON ADRIATIC FIGS, 1944

Date	Number		Average number of eggs per scale
	Scales examined	Eggs found	
July 17.	60	692	11.5
July 24...	50	643	12.8
August 1..	40	508	12.7
August 7.	40	505	12.6
Total...	190	2,348	12.4

Eggs of First Summer Brood Scale. Observations on the number of eggs laid by the first summer brood were made in the same manner as for the overwintered females. The observations are summarized in table 6. The range was from 0 to 23, and the average was 12.4 eggs per female—only 40 per cent as great as the average for the overwintered females. These data approach the figures 10.9 average and 24 maximum presented by Simmons *et al.*, in 1931.

In addition to the fact that individual summer scales are smaller than overwintering scales, there is a difference in body size and number of eggs produced between scales on the upper and lower surfaces of the leaves. On July 3, 1944, 21 scales from the upper surface showed an average of 12.7 eggs per scale, while 17 scales from the rough pubescent lower leaf surface showed an average of 7.2 eggs per scale.

Observations on the hatching period of the second summer brood of the scale were made in the same manner as for the first brood. The period could be clearly traced for about 4 weeks. As previously noted, hatching of the second brood began between the middle and the twenty-fourth of June, before hatching of the first brood was complete. Hatching was not in active progress until after July 3 for, as shown in table 7, only about 1 per cent of the eggs observed on June 24 and July 3 had hatched. During the 4 weeks—July 3 to August 1—the percentage of hatched eggs increased uniformly. Observations were not made after August 7.

Location of Scale while Trees Are in Foliage. The location of scale on the current growth (1944) was studied on eight occasions by examination of foli-

age, second crop fruit, and current wood from six locations on a heavily infested tree. The six locations on the tree were the upper and lower center parts and the northwest, northeast, southwest, and southeast sides of the outside crown. No record was made of the number of live and dead scales. The observations are summarized in table 8.

TABLE 7
HATCHING OF SECOND BROOD FIG SCALE FROM
FIRST SUMMER BROOD EGGS, 1944

Date	Number		Per cent of eggs hatched	Per cent of scale with hatching complete
	Scales examined	Eggs observed		
June 24.	100	688	1 3	0.0
July 3.	38	348	0.6	0.0
July 17.	60	692	44 7	1.7
July 24.	50	643	59 6	10 0
August 1.	40	508	79.7	20 0
August 7.	40	505	60.2	2.5

TABLE 8
NUMBER OF SCALES AND PERCENTAGE OF POPULATION FOUND ON 1944 WOOD,
FRUIT, AND FOLIAGE OF SIX TWIG SAMPLES

Date	Number of scales on				Average number of scales per fig	Total number of scales counted	Per cent of scale on			
	Wood	Petioles	Leaves	Fruit			Wood	Petioles	Leaves	Fruit
June 7.	29	44	8,073	23	1.0	8,169	0 4	0 5	98.8	0.3
27.	20	62	9,468	2	1.0	9,552	0 2	0.7	99 1	0.2
July 10.	23	63	8,227	230	17.7	8,543	0 3	0.7	96 3	2.7
17.	66	147	8,950	345	18.2	9,508	0 7	1.6	94 1	3.6
24.	56	95	10,625	452	19.8	11,226	0 5	0 8	94.6	4.0
Aug. 1.	62	137	9,495	503	22.9	10,197	0.6	1.3	93 1	4 9
7.	10	173	20,826	358	17.1	21,367	0 1	0.8	97.5	1.8
14.	22	171	21,586	270	15 0	22,049	0.1	0 8	97 9	0.1
Total	288	892	97,248	2,183		100,611	0 3	0.9	96.7	2.2

The data show that from June to mid-August more than 90 per cent of the scales on the current season's growth was found on the leaves. Observations made early in the season in 1945 show that this situation also prevailed in late April and early May. On April 23, May 2, and May 9, 1945, the per cent of total population found on the leaves was 88.5, 98.9, and 96.3, respectively. Nearly all of the first brood of males had emerged by mid-July so that count of scales on leaves made at later dates included a great many empty male scales. Further, the data does not distinguish between live and dead scales, and since other observations showed that the proportion of dead scales increases on the leaves as the season progresses, table 8 does not serve as a record of the number of live scales present after mid-July.

Casual observations by the writers, certain fig growers, and agricultural extension workers indicated that scale infestation was often heaviest on the northwest portion of the tree. To augment these casual observations, the density of scale population on different parts of the tree was determined by an examination of foliage from six locations on five Adriatic trees on twelve dates from June 27 through September 18. On the trees selected, which were separated from adjoining trees by several feet, the well-established scale had been uncontrolled for several years. The samples were taken from the same locations in the trees as the samples used to study location of summer brood scales.

TABLE 9

NUMBER OF SCALES RECORDED ON THIRD OLDEST LEAVES OF 1944 GROWTH AT SIX STATIONS ON FIVE ADRIATIC FIG TREES FROM JUNE 27 THROUGH SEPTEMBER 18

Date	Number of scales recorded at following parts of 5 trees						Total number of scales counted	Average per leaf
	Upper center	Lower center	North-west	North-east	South-east	South-west		
June 27.....	1,543	553	2,286	1,147	1,564	1,055	8,148	271.6
July 10.....	782	1,081	1,727	1,365	987	644	6,586	219.5
17.....	554	1,808	2,226	1,677	1,507	800	8,572	285.7
24.....	719	1,505	1,761	1,346	1,460	1,035	7,826	260.8
August 1.....	866	1,237	1,856	1,567	1,339	766	7,631	254.3
7.....	2,835	3,979	2,554	2,747	2,573	1,493	16,181	539.3
14.....	1,748	3,375	4,040	3,790	3,609	2,522	19,082	636.0
21.....	2,120	4,586	3,612	2,704	3,286	2,766	19,074	635.8
28.....	2,725	2,443	4,138	2,224	3,435	2,796	17,761	592.0
September 4.....	2,322	3,355	5,213	3,268	5,071	3,910	23,139	771.3
11.....	3,161	3,247	3,641	2,855	4,145	3,477	29,526	984.2
18.....	2,964	4,326	5,540	3,535	7,455	4,821	28,641	954.7
Total.....	22,339	31,495	38,594	28,225	36,431	26,085	183,167	
Average per leaf.....	372.4	525.0	643.4	470.5	607.3	434.8		508.8

Observations consisted of recording the number of scales on the third oldest leaf of each twig sample. They are summarized in table 9. Counts were made without the aid of magnification, and number of live and dead scales was not determined.

The average population densities on the northwest and southeast sides of the trees were significantly greater statistically than those for other locations on the trees. The writers suggest that differences in temperature may cause the differences in population densities in various locations. The lower center and northeast portions of the tree thus may have temperatures below the optimum necessary for crawlers to settle and become established—especially during late August and September. On the other hand, the upper center and southwest portions of the tree may have temperatures too high to be attractive to crawlers. The northwest and southeast portions of the tree seem to have the most attractive temperatures, the southeast appearing better than the northwest in late August and September. Between the dates of August 1 and 7, an increase in general abundance was indicated, the average per leaf increasing from 254.3 to 539.3.



Fig. 12.—Relative abundance of adult female fig scale without eggs at different locations on the tree in 1945.

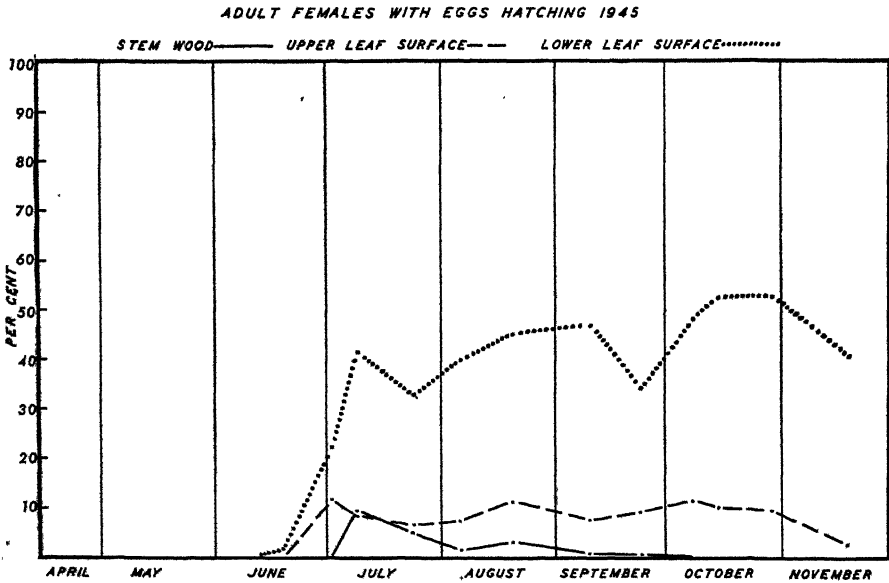


Fig. 13.—Relative abundance of adult female fig scales with eggs hatching at different locations on the tree in 1945.

On June 7, a few fruits were found to be infested with young scales. This fruit infestation must have resulted from crawlers hatched from eggs laid by overwintered scales, since the hatching of eggs laid by the first summer brood scales did not begin until mid-June. From July 10 to August 14, when the observations were discontinued, the number of scales per fruit was enough to make the fruit unattractive. Observations on leaf petioles in 1944 and on fruit in 1945 showed a rapidly increasing infestation during the latter part of August and the month of September. An increase in the rate of production of crawlers and their movement from the leaves was indicated.

Observations on the location of female scales on new wood were made between October 2 and November 20, 1944. The area of the bark examined was

TABLE 10
NUMBER OF LIVING FEMALE SCALES PER SQUARE INCH OF NEW WOOD
ON SIX TWIGS BETWEEN OCTOBER 2 AND NOVEMBER 20, 1944

Date	Number of scales	
	Observed	Per square inch
October 2..	2,405	75.6
October 9	1,709	62.2
October 16	1,616	59.4
October 23	826	32.3
October 30.. . . .	817	30.2
November 7.....	881	43.6
November 13.....	533	20.3
November 20	385	12.4
Total.	9,172	

estimated, and the number of live female scales per square inch was recorded. The results summarized in table 10 show that the observations were begun after the peak of infestation had been reached. The sharp decrease in infestation was due largely to predation. In the following year, observations on number of live male and female scales on the new wood were started in April and continued until November 19. These observations were made from a group of about ten trees, with twigs from the same six sampling locations used for the 1944 examinations. The results are summarized in table 11. It was shown that in August a rapid increase in infestation reached a peak on September 10. In October, a sharp decrease in infestation occurred, similar to that which had occurred the previous year. This decrease in 1945 was again largely attributable to predation.

Natural Enemies. The predators which seemed most active during the fall were the armored scale predator, *Lindorus lophanthæ* (Blaisdell), the two-stabbed ladybird beetle, *Chilocorus bivulnerus* Muls., and lacewing larvae. On January 25 and February 2 of 1945 a total of 4,352 scales was examined on the youngest wood. Of these, 2,872, or 66.1 per cent, were dead or empty. Practically all of this mortality was due to predation. Among the 1,377 scales not killed by predators, 21, or 1.4 per cent, were parasitized by a small hymenopterous parasite, *Aphytis mytilaspidis* (LeBaron) and probably also by

Aphytis chrysomphali (Mercet) which had been reported by other workers in 1943. All but 1 or 2 of the parasitized scales were still alive.

Counts of 1,800 scales each were made on January 31, February 14, March 15, and April 10 of 1944. Only scales which appeared well developed were examined. Empty scales which had resulted from attacks of predators of one kind or another were discarded. An average of 5 per cent of the scales was found to be dead.

On February 28, 1945, a similar count of 2,000 scales was made, and 6.9 per cent was found to have died from causes other than predation. Consider-

TABLE 11
NUMBER OF LIVING SCALES ON NEW WOOD OF SIX
TWIGS BETWEEN APRIL 23 AND NOVEMBER 19, 1945

Date	Number of scales
April 23.	50
May 2	10
May 9.	37
May 14.	13
May 21.	23
May 28.	18
June 4	10
June 11.	13
June 19.	9
July 2.	16
July 9.	42
July 24.	81
August 1.	419
August 20.	535
September 10	1,986
September 24.	1,656
October 8	1,953
October 15	784
October 29.	1,071
November 19.	803
Total	11,529

ing the 1944 fall observations, together with the 1945 early winter observations, 66.1 per cent of the settled population of the new wood failed to establish itself for the winter. By February 28, additional mortality accounted for 2.3 per cent more of the original settled population.

In the spring and summer other predators have been noted. One is the slow-moving mite of the *Hemisarcoptes* species which lives under the scales and feeds on the scale eggs and often on the female bodies. A much larger and more active mite of *Seiulus* species has been seen feeding on the crawlers and the very young settled scales.

Although the natural enemies of the fig scale do noticeably reduce the overwintering population, the reduction is insufficient to check the scale. Where control measures have not been applied, the scale has generally increased to damaging proportions.

Relative Abundance of Different Developmental Stages during the Year.

In June of 1944, observations were begun on the life history of the fig scale in the field. Approximately weekly observations were made to determine

about what proportion of the various stages of development one might expect to find in the field on any particular date. Single twig samples were examined from the upper center, lower center, north, east, south, and west portions of the trees. Usually six different trees were used to supply the six location samples on any one sampling date. Separate records were made of insects on the upper and lower leaf surfaces, and on current stem wood. The number of scales examined depended on the relative abundance of live insects at the various locations. The developmental stages were divided into nine categories: 1) first settled stage before first moult; 2) insects in the process of first moult; 3) insects having passed the first molt, but too young to show sex differentiation; 4) second stage females; 5) second stage males and male pupae; 6) females in process of second moult; 7) adult females without eggs; 8) females laying eggs which had not begun to hatch; and 9) females with eggs hatching. The results of these examinations are summarized in tables 12 and 13.

Five of these developmental stages for the years 1944 and 1945 are shown graphically (figures 4 to 13). In all these graphs the solid line represents the scales on the stem wood; the bar line, the scales on the upper surfaces of the leaves; and the dotted line, the scales on the lower surfaces of the leaves. The lines represent the per cent of the live developmental stage on these locations. For example, figure 9 shows that in early July, 1945, about 25 per cent of all the scales examined on the current stem wood were in the first settled stage of development. At the same time, about 10 per cent examined on both upper and lower leaf surfaces were in the first settled stage of development.

In general, the graphs show two peaks of relative abundance for each developmental stage. Observations were begun so late in 1944 that, in most instances, the graphs for that year show only the second peaks. For the first settled stage (figures 4 and 9) the first peak was distinct while the second peak was much less so, especially on the leaves in 1945. The first peak for the early second stage (fig. 10) was not so distinct as that for the first settled stage. The second peaks for the early second stage (figures 5 and 10) were especially distinct for the upper leaf surface and stem wood. Figures 6 and 11 show, as previous workers have noted, that in the first brood up to July the proportion of males on the upper surfaces of the leaves was greater than on the lower surfaces. Just the reverse was true of the females (figures 7 and 12). Later in the season, however, a large percentage of the males settled on the lower leaf surfaces (figures 7 and 12). A superficial examination would not reveal this fact, since most of the empty shells remain on the upper surfaces of the leaves after the emergence of males.

The second peak of relative abundance of male scales occurred in late September in 1944 and 1945. The steady decrease in the percentage of males on the leaves in October and early November is explained by the emergence of adult males. At the same time that the males emerged, a rapid rise in the percentage of young adult female scales on the stem wood (figures 7 and 12) occurred. These became the overwintering generation. The fact that the males were emerging at the time the females were maturing indicated that the females must overwinter in a fertilized condition. In fact, mating on the stem wood has been repeatedly observed in October and early November, but males have not been found in the winter or early spring.

TABLE 12

FIG SCALE ON ADRIATIC FIGS, FRESNO, 1944

(Stage of development as per cent of total scales examined on each location for each date)

Date	Location	First settled stages	In process of first moult	Early second stage	Second stage, female	Settled stages, male	Females in process of second moult	Females without eggs	Females with eggs not hatching	Females with hatching eggs	Total number scales examined
June 10, 1944	Stem wood	15.4	21.4	8.6	5.1	5.9	13.7	30.0	0.0	0.0	117
	Upper leaf surface	8.2	14.8	24.8	1.6	34.9	4.2	10.3	1.2	0.0	243
	Lower leaf surface	15.4	4.2	5.4	7.6	3.7	10.0	43.3	10.4	0.0	240
June 17, 1944	Stem wood	20.1	14.8	17.7	4.1	5.4	8.3	27.8	1.8	0.0	169
	Upper leaf surface	10.1	15.1	17.0	7.4	27.1	3.6	14.7	5.0	0.0	217
	Lower leaf surface	8.4	5.6	.9	5.6	2.8	11.2	40.2	24.8	0.5	214
June 24, 1944	Stem wood	16.7	16.1	17.7	8.3	2.1	11.5	23.4	4.2	0.0	192
	Upper leaf surface	6.9	10.4	26.0	3.9	23.5	5.4	12.7	8.3	2.9	204
	Lower leaf surface	1.0	2.0	2.9	6.9	0.5	11.3	23.5	47.5	4.4	204
July 1, 1944	Stem wood	34.6	6.7	13.9	2.4	3.0	4.8	20.6	9.2	4.8	165
	Upper leaf surface	35.6	23.3	7.3	5.0	9.1	3.2	7.3	2.3	6.8	219
	Lower leaf surface	10.6	2.3	0.5	0.0	0.0	1.9	29.7	18.0	37.0	216
July 10, 1944	Stem wood	52.2	9.0	10.7	4.5	2.2	5.1	10.7	2.2	3.4	178
	Upper leaf surface	28.9	21.2	11.4	3.3	7.6	4.3	11.4	4.3	7.6	211
	Lower leaf surface	8.0	2.4	1.9	1.9	0.0	4.3	21.8	9.0	50.7	211
July 15, 1944	Stem wood	51.8	20.3	8.1	3.0	1.0	2.0	9.1	1.0	3.6	197
	Upper leaf surface	24.3	27.7	20.7	1.0	9.9	1.0	9.9	3.0	2.5	202
	Lower leaf surface	8.9	7.0	3.0	5.4	1.0	3.5	16.9	9.0	45.3	201
July 24, 1944	Stem wood	41.8	20.6	20.6	1.3	0.4	2.6	5.7	1.3	5.7	228
	Upper leaf surface	23.1	25.3	15.6	4.3	7.6	3.8	9.7	5.4	5.4	186
	Lower leaf surface	2.7	6.5	6.5	4.8	0.5	5.9	34.3	9.8	20.0	186
August 1, 1944	Stem wood	34.3	38.4	16.2	3.3	0.0	1.2	4.2	.4	2.0	240
	Upper leaf surface	20.6	26.0	20.6	3.9	8.3	6.7	8.9	1.1	3.9	180
	Lower leaf surface	6.7	7.8	3.9	5.6	2.8	11.0	17.2	4.4	40.6	180

August 7, 1944	Stem wood.....	28.7	27.5	37.6	2.5	.4	.4	1.7	.4	8	240
	Upper leaf surface.....	10.0	27.8	27.2	5.6	4.4	3.3	13.9	2.8	5.0	180
	Lower leaf surface.....	2.2	6.0	12.8	3.9	2.2	5.6	28.8	7.8	31.7	180
August 14, 1944	Stem wood.....	26.1	18.8	47.8	1.7	.4	1.3	2.2	0.0	1.7	230
	Upper leaf surface.....	10.7	17.2	34.5	3.9	3.9	6.5	16.8	2.7	3.8	185
	Lower leaf surface.....	1.6	3.9	12.4	2.7	1.6	6.5	34.0	7.0	30.3	185
August 22, 1944	Stem wood.....	26.0	18.2	32.9	18.6	.4	0.0	2.6	.4	9	231
	Upper leaf surface.....	10.3	20.5	26.8	1.1	3.8	4.8	13.5	4.9	3.8	185
	Lower leaf surface.....	.5	6.0	6.5	3.8	1.6	6.5	26.7	10.9	37.5	184
August 23, 1944	Stem wood.....	15.5	15.5	45.5	21.2	0.0	1.2	4	0.0	4	240
	Upper leaf surface.....	10.6	13.8	44.4	6	10.6	2.8	6.7	1.1	4.4	180
	Lower leaf surface.....	.6	2.8	6.1	2.8	3.3	2.2	19.4	8.9	53.9	180
September 4, 1944	Stem wood.....	16.7	16.6	22.8	33.3	.4	7.9	2.1	0.0	1.2	240
	Upper leaf surface.....	8.8	16.8	33.0	.6	25.1	.6	7.8	3.4	3.9	179
	Lower leaf surface.....	6	1.1	5.5	3.3	11.1	2.2	19.9	14.9	41.4	181
September 10, 1944	Stem wood.....	19.2	13.7	17.1	33.8	1.2	12.1	2.1	0.0	6	240
	Upper leaf surface.....	15.0	22.2	22.8	0.0	30.6	2.2	0.0	3.9	3.3	180
	Lower leaf surface.....	.6	2.8	3.9	.6	12.2	1.1	12.2	10.6	56.0	180
September 18, 1944	Stem wood.....	15.4	19.6	17.9	28.2	0.0	11.2	7.1	0.0	.6	240
	Upper leaf surface.....	8.9	22.3	14.5	0.0	44.2	2.8	1.7	1.1	4.5	179
	Lower leaf surface.....	0.0	5.5	8.3	0.0	18.3	1.1	7.7	6.1	53.0	181
September 26, 1944	Stem wood.....	14.6	12.9	18.3	15.0	0.0	19.6	19.6	0.0	0.0	240
	Upper leaf surface.....	18.1	23.9	18.3	5	31.7	1.1	1.7	1.7	5.0	180
	Lower leaf surface.....	0.0	3.3	5.6	0.0	19.4	1.1	2.2	3.9	64.5	180
October 2, 1944	Stem wood.....	6.0	13.9	13.3	18.8	0.0	16.0	32.3	0.0	7	300
	Upper leaf surface.....	9.3	23.3	16.0	.7	42.7	2.7	1.3	0.0	4.0	150
	Lower leaf surface.....	2.7	5.3	1.3	0.0	27.4	0.0	2.7	1.3	59.3	150
October 9, 1944	Stem wood.....	6.1	8.0	9.7	13.9	.3	20.0	41.1	.3	.6	360
	Upper leaf surface.....	14.2	18.2	16.7	0.0	44.2	.8	1.7	0.0	4.2	120
	Lower leaf surface.....	3.3	0.0	4.2	0.0	21.6	.8	1.7	4.2	64.2	120

TABLE 12—Continued

Date	Location	First settled stages	In process of first moult	Early second stage	Second stage, female	Settled stages, male	Females in process of second moult	Females without eggs	Females with eggs not hatching	Females with hatching eggs	Total number scales examined
October 15, 1944	Stem wood	6.9	6.7	8.3	10.0	0.0	20.0	48.1	0.0	0.0	390
	Upper leaf surface	14.2	20.8	20.0	.8	32.6	.8	7.5	0.0	3.3	120
	Lower leaf surface	3.3	0.0	2.5	0.0	20.0	0.0	2.5	0.0	71.7	120
October 22, 1944	Stem wood	4.5	8.1	8.6	10.9	0.0	13.6	54.3	0.0	0.0	396
	Upper leaf surface	13.6	24.5	10.8	0.0	41.2	1.0	2.9	0.0	1.0	102
	Lower leaf surface	2.9	1.0	2.0	0.0	15.7	1.0	2.9	1.0	73.5	102
October 30, 1944	Stem wood	1.3	2.0	4.5	6.3	0.0	12.4	73.0	0.0	5	306
	Upper leaf surface	10.9	33.7	17.8	0.0	20.7	2.0	3.0	1.0	10.9	101
	Lower leaf surface	7.8	11.7	2.9	0.0	7.8	9.7	2.9	0.0	57.2	103
November 6, 1944	Stem wood	3.0	3.3	1.5	7.1	0.0	10.1	75.0	0.0	0.0	396
	Upper leaf surface	6.9	24.5	24.5	4.9	28.4	5.9	2.9	0.0	2.0	102
	Lower leaf surface	5.9	5.9	6.9	3.9	13.7	2.0	4.9	1.0	55.8	102
November 22, 1944	Stem wood	0.0	1.0	5	1.5	0.0	5.8	91.2	0.0	0.0	306
	Upper leaf surface	1.0	24.7	36.7	2.0	9.9	7.9	11.9	0.0	5.9	101
	Lower leaf surface	3.9	6.8	6.8	1.9	3.9	2.9	2.9	1.9	69.0	103
November 29, 1944	Stem wood	0.0	0.0	.2	7	0.0	3.9	95.2	0.0	0.0	440
	Upper leaf surface	0.0	11.8	11.8	5.9	17.5	5.9	41.2	0.0	5.9	17
	Lower leaf surface	6.7	6.7	20.0	13.3	0.0	6.7	26.6	0.0	20.0	15

Hatching of eggs of the first summer brood females was first observed on June 17, 1944 and on June 19, 1945 (figures 8 and 13). Beginning in late June, there was a rapid rise in the per cent of females with hatching eggs on the lower surfaces of the leaves. The percentage of females with hatching eggs was low on the stems (under 10 per cent) and upper leaf surfaces (under 12 per cent) for the entire season.

In contrast, the percentage of females with hatching eggs on the lower leaf surfaces remained above 30 per cent until observations were discontinued in November. Two possible explanations are suggested for this latter occurrence: 1) female crawlers settle on the lower leaf surfaces over a long period in the spring where they remain alive for three or four months; and 2) a certain portion of the female progeny of the first summer brood females may settle on the leaves. The writers believe the second explanation to be the more logical one. The data then show an overwintering brood of fertilized females, a summer brood of widely overlapping developmental stages, and a partial late summer or early fall brood. The writers suggest that the first progeny of the summer brood forms the partial second summer brood, and that the later progeny, together with the progeny of the second summer brood, become the overwintering generation.

These data in themselves constitute strong evidence that only one species of fig scale was involved. Just before growth of the fig tree started in the spring, the scale population was—so far as could be observed—composed entirely of *ficus*-form females. As soon as the leaves began to grow, the eggs of these females began to hatch, and a *ficifoliae*-form female infestation appeared on the leaves. Increased infestation on both new and old wood was negligible.

In early August all the overwintered females were dead. Very few adult *ficus*-form females could be found on the twig wood and none was observed on the leaves. Yet it was in August that large numbers of crawlers began to settle on the twig wood and later became overwintering *ficus*-form females.

Location of *Ficus*- and *Ficifoliae*-Form Females. In the winter of 1944–1945 all the females that were taken from the wood, mounted, and examined under the microscope were observed to be the *ficus* form, as illustrated by Ferris (1937, 1938). In the winter of 1943–1944 the writers observed on rare occasions one or two females per twig that were about the size and color of the *ficifoliae*-form female. These scales were usually found near the terminal buds. In contrast to the overwintering *ficus* form in midwinter, all of these smaller, narrower, lighter-colored females contained eggs, a majority of which had already hatched. Unfortunately, none of these scales was mounted for microscopic examination. Live *ficus*-form females could be found on the previous season's wood growth as late as mid-June.

Based on the shape of the pygidial lobes, both *ficus*- and *ficifoliae*-form females were found on the youngest twig growth in mid-July. The presence of elongated scleroses arising from the base of the median lobes on the ventral side is the "key character" given by Ferris for the separation of the two forms. Where the lobes were *ficifoliae* shaped these scleroses were always present, but where the pygidial lobes were *ficus* shaped no such scleroses were observed. On the current twig wood in July, the adult females with *ficus*-shaped lobes were smaller and a lighter brown than the overwintering scales.

TABLE 13

FIG SCALE ON ADRIATIC FIGS, FRESNO, 1945

(Stage of development as per cent of total scale examined on each location for each date)

Date	Location	First settled stages	In process of first moult	Early second stage	Second stage, female	Settled stages, male	Females in process of second moult	Females without eggs	Females with eggs not hatching	Females with hatching eggs	Total number scales examined
April 8, 1945	Stem wood	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1
	Upper leaf surface	0
	Lower leaf surface	0
April 16, 1945	Stem wood	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1
	Upper leaf surface	0
	Lower leaf surface	0
April 23, 1945	Stem wood	96.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50
	Upper leaf surface	47.2	45.8	7.0	0.0	0.0	0.0	0.0	0.0	0.0	288
	Lower leaf surface	33.3	29.2	37.5	0.0	0.0	0.0	0.0	0.0	0.0	96
May 2, 1945	Stem wood	50.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10
	Upper leaf surface	36.8	51.8	11.2	0.2	0.0	0.0	0.0	0.0	0.0	529
	Lower leaf surface	64.4	24.0	9.6	1.1	0.9	0.0	0.0	0.0	0.0	363
May 9, 1945	Stem wood	29.9	35.0	18.9	2.7	10.8	2.7	0.0	0.0	0.0	37
	Upper leaf surface	8.3	34.9	28.8	3.4	24.4	0.2	0.0	0.0	0.0	525
	Lower leaf surface	20.7	28.6	22.4	10.6	10.6	7.1	0.0	0.0	0.0	434
May 14, 1945	Stem wood	7.7	7.7	46.1	0.0	30.8	0.0	7.7	0.0	0.0	13
	Upper leaf surface	0.8	4.0	19.2	0.0	72.8	3.2	0.0	0.0	0.0	125
	Lower leaf surface	2.4	0.8	5.7	35.0	14.6	29.3	12.2	0.0	0.0	123
May 21, 1945	Stem wood	34.8	4.3	8.7	8.7	39.2	4.3	0.0	0.0	0.0	23
	Upper leaf surface	1.6	9.6	19.2	0.0	69.6	0.0	0.0	0.0	0.0	125
	Lower leaf surface	1.9	2.8	13.2	23.7	19.8	31.1	7.5	0.0	0.0	106
May 28, 1945	Stem wood	38.9	11.1	11.1	11.1	16.7	11.1	0.0	0.0	0.0	18
	Upper leaf surface	4.1	7.6	18.6	0.0	67.6	2.1	0.0	0.0	0.0	145
	Lower leaf surface	11.8	2.1	16.0	5.6	20.1	34.7	9.7	0.0	0.0	144

June 4, 1945	Stem wood.....	10.1	0.0	0.0	70.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	10
	Upper leaf surface.....	1.4	0.7	12.5	0.0	78.4	2.1	4.9	0.0	0.0	0.0	0.0	144
	Lower leaf surface.....	3.5	1.4	5.7	5.0	10.6	6.4	63.9	3.5	0.0	0.0	0.0	141
June 11, 1945	Stem wood.....	7.8	23.1	0.0	0.0	15.3	23.1	30.7	0.0	0.0	0.0	0.0	13
	Upper leaf surface.....	0.0	0.8	5.5	0.0	86.7	0.0	6.2	0.8	0.0	0.0	0.0	128
	Lower leaf surface.....	1.6	0.0	1.6	4.8	4.8	3.2	61.8	22.2	0.0	0.0	0.0	126
June 19, 1945	Stem wood.....	33.3	11.1	0.0	0.0	33.3	0.0	22.3	0.0	0.0	0.0	0.0	9
	Upper leaf surface.....	2.7	3.7	14.7	0.0	56.0	0.0	8.2	14.7	0.0	0.0	0.0	109
	Lower leaf surface.....	2.3	0.0	4.6	0.8	3.1	6.2	44.6	36.9	1.5	0.0	0.0	130
July 2, 1945	Stem wood.....	25.0	37.6	0.0	0.0	0.0	6.2	25.0	6.2	0.0	0.0	0.0	16
	Upper leaf surface.....	11.6	18.8	17.4	0.0	27.6	0.0	4.3	8.7	11.6	0.0	0.0	69
	Lower leaf surface.....	10.1	1.8	0.0	0.9	5.5	4.6	24.8	30.3	22.0	0.0	0.0	109
July 9, 1945	Stem wood.....	50.0	19.0	11.9	4.8	0.0	2.4	2.4	0.0	0.0	0.0	0.0	42
	Upper leaf surface.....	12.8	25.8	42.2	1.8	6.4	1.8	0.9	0.0	0.0	0.0	0.0	109
	Lower leaf surface.....	13.9	10.5	11.4	3.5	4.3	1.7	7.8	5.2	41.7	0.0	0.0	115
July 24, 1945	Stem wood.....	30.9	22.2	24.7	2.5	0.0	2.5	12.3	0.0	0.0	0.0	0.0	81
	Upper leaf surface.....	9.7	7.5	26.8	1.1	33.3	4.3	9.7	1.1	0.5	0.5	0.5	93
	Lower leaf surface.....	2.2	1.1	7.6	7.6	6.5	7.6	33.7	1.1	32.6	0.0	0.0	92
August 6, 1945	Stem wood.....	21.5	26.7	36.5	8.1	1.7	0.2	2.9	1.0	1.4	0.0	0.0	419
	Upper leaf surface.....	2.4	8.4	25.4	1.2	28.9	1.2	14.5	10.8	7.2	0.0	0.0	83
	Lower leaf surface.....	2.4	3.6	9.6	0.0	4.8	2.4	20.5	16.9	39.8	0.0	0.0	83
August 20, 1945	Stem wood.....	23.4	21.8	26.0	19.2	.2	.9	3.2	2.1	3.2	0.0	0.0	535
	Upper leaf surface.....	11.3	18.3	28.7	1.7	17.4	0.0	5.2	6.1	11.3	0.0	0.0	115
	Lower leaf surface.....	7.8	4.3	7.8	7.8	13.1	0.0	4.3	9.7	45.2	0.0	0.0	115
September 10, 1945	Stem wood.....	8.8	29.6	19.6	24.5	.1	14.6	2.0	0.0	.8	0.0	0.0	1,986
	Upper leaf surface.....	3.2	5.3	20.0	3.2	57.8	0.0	3.2	0.0	7.3	0.0	0.0	95
	Lower leaf surface.....	3.1	4.2	5.2	8.3	27.1	0.0	2.1	3.1	46.9	0.0	0.0	96

TABLE 13—Continued

Date	Location	First settled stages	In process of first moult	Early second stage	Second stage, female	Settled stages, male	Females in process of second moult	Females without eggs	Females with eggs not hatching	Females with hatching eggs	Total number scales examined
September 24, 1945	Stem wood.....	8.5	13.1	13.8	16.7	0.0	27.8	19.4	0.0	.7	1,650
	Upper leaf surface.	2.0	10.0	22.0	2.0	54.0	1.0	0.0	0.0	9.0	100
	Lower leaf surface.....	1.0	5.0	9.0	4.0	46.0	0.0	0.0	2.0	34.0	101
October 8, 1945	Stem wood.	2.0	4.6	4.4	13.0	3	19.5	56.0	0.0	0.2	1,953
	Upper leaf surface.9	15.2	19.6	0.0	49.1	0.0	3.6	0.0	11.6	112
	Lower leaf surface.....	4.4	3.6	4.5	0.0	36.6	0.0	2.7	0.0	48.2	112
October 15, 1945	Stem wood.....	0.0	8.4	8.7	9.2	2	11.9	61.6	0.0	0.0	784
	Upper leaf surface.....	.8	10.8	21.7	1.7	51.7	0.0	3.3	0.0	10.0	120
	Lower leaf surface.....	0.0	.8	9.2	1.7	33.3	0.0	1.7	.8	52.5	120
October 20, 1945	Stem wood.....	0.0	.1	.4	9.4	0.0	6.9	83.2	0.0	0.0	1,071
	Upper leaf surface.	2.6	16.4	15.5	0.0	43.1	1.7	11.2	0.0	9.5	116
	Lower leaf surface.....	.9	8.6	6.9	.9	24.1	1.7	4.3	0.0	52.6	116
November 19, 1945	Stem wood...	0.0	0.0	0.0	2.5	0.0	1.6	95.9	0.0	0.0	803
	Upper leaf surface.....	0.0	6.2	16.2	5.0	23.8	0.0	46.3	0.0	2.5	80
	Lower leaf surface	2.7	5.4	13.5	0.0	4.1	8.1	25.7	0.0	40.5	74

On the leaves through spring and summer until October, all female scales, either in external appearance or when mounted and examined under the microscope, were observed to be *ficifoliae* form. After October, both *figus*-form and *ficifoliae*-form females were found on the leaves, the former easily distinguished by their wider, darker-colored coverings. Nearly all of the *ficifoliae*-form females on the leaves in October were laying eggs. *Figus*-form females on the leaves had never been observed to be laying eggs until October 1, 1946. On that date, females with *figus*-shaped lobes were found to be laying eggs under scales that were only slightly larger and darker than the typical *ficifoliae* form.

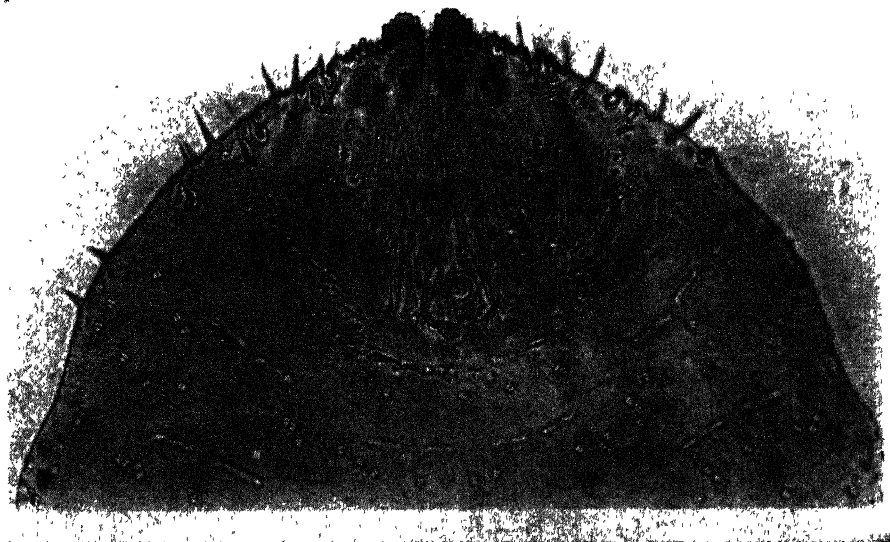


Fig. 14.—Pygidium of *figus*-form female fig scale. (Enlarged about 330 times.)

The writers have never found a heavy midsummer infestation of *ficifoliae*-form females on the leaves without finding the empty scales of overwintered *figus* females on the wood of the previous season.

Egg Transfer Experiments. In the spring of 1945 many experiments were made in which eggs from a single overwintered female were transferred to a small cellophane cage fastened with paraffin to a fig leaf. After the transfer, the cage was closed by means of a glass coverslip fastened with paraffin to the cage. The female body was mounted on a glass slide. The eggs hatched but only males developed.

In the fall of 1945, eggs from several females taken from fig leaves were transferred to a fig leaf on an uninfested isolated fig plant. The section of the stem bearing this leaf was isolated by two tanglefoot bands. The procedure was repeated with a second leaf on the plant. The female bodies were mounted on glass slides. Subsequent examination showed all to be of typical *ficifoliae* form. Both *figus*- and *ficifoliae*-form females developed on the leaf surface. Only *figus*-form females appeared on the leaf petioles and stem wood.

Egg transfers from overwintered *ficus*-form females were again made in the spring of 1946. Eggs from a single female were transferred to a leaf on an uninfested plant, and the female body was mounted on a glass slide (fig. 14). The part of the stem bearing the leaf was isolated with tanglefoot bands. Typical *ficifoliae*-form females (confirmed by microscopic examination of mounted specimens) developed on the leaves (fig. 15). These experiments are a confirmation of the work of Lupo (1943), who first stated that *Lepidosaphes ficifoliae* was but a summer form of *L. ficus*.

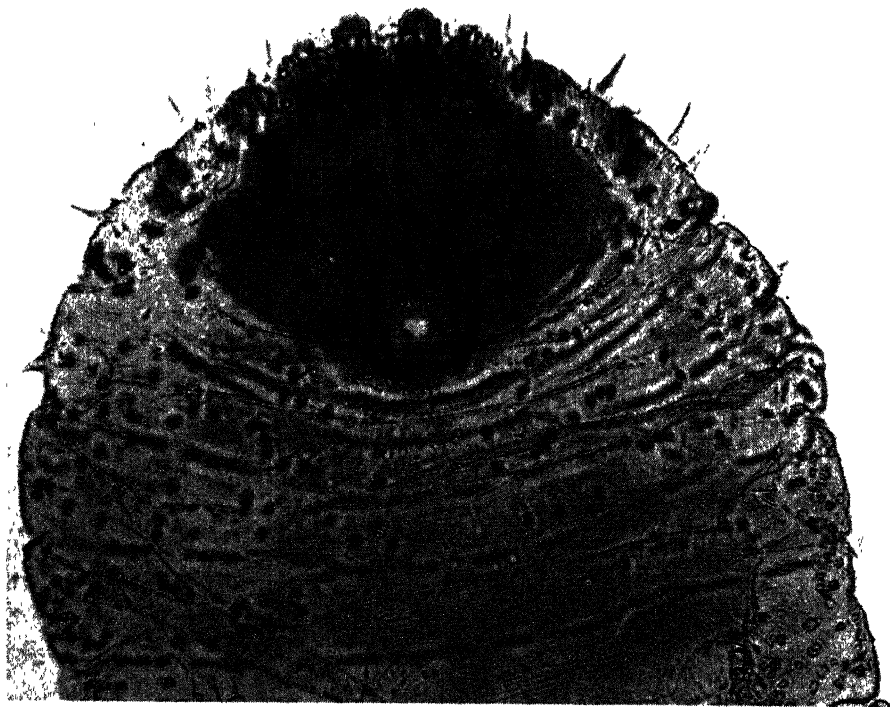


Fig. 15.—Pygidium of *ficifoliae*-form scale. This female was one of the progeny of the female pictured in figure 14. (Enlarged about 330 times.)

SUMMARY

The fig scale, long a pest of cultivated figs in the Mediterranean area, was probably brought to California in 1905. The insect now occurs from Tulare County in the south to San Joaquin County in the north central part of the state. An isolated infestation occurs in Glenn County.

The warty appearance of infested dried figs is unsightly. Heavily infested figs are small, shriveled, and light in weight. The dark green spots beneath scales on ripe canning figs remain after processing. The presence of these scale marks lowers the ripe figs from top, or canning grade, to jam grade, with consequent reduction in value.

The winter of the scale is spent as fertilized adult females. By far the greatest percentage of live scale is found on the one- and two-year-old wood, but

live scale may occur on succulent bark on any part of the tree above the ground. Only 60 to 70 per cent of the scales on the dormant trees contain live females.

Egg laying by overwintering females starts in February, the scale on older wood and trunk being first to oviposit. About 20 per cent of the living scales have begun to lay eggs when apricots begin to bloom, and about 90 per cent are laying eggs when the apricot bloom is falling. At this time the buds on the side branches of Adriatic fig trees have elongated from $\frac{1}{2}$ to 1 inch. On the average, each overwintered female lays about 30 eggs. Hatching starts around the first of April, and egg laying and hatching continue so that the crawlers from overwintered females are produced into late June. In 1944, eggs were present from February 7 to June 24.

The male crawlers from the overwintered female eggs settle mostly on the upper surfaces of the leaves, the female crawlers largely on the lower leaf surfaces. Well over 90 per cent of all crawlers settle on the leaves, less than 2 per cent on the leaf petioles, less than 1 per cent on the new wood growth, and about 2 per cent on the second crop figs.

Female scales found on the leaves throughout the summer are shorter, narrower, and lighter colored than the overwintering form. On the upper leaf surfaces the female scales are somewhat larger and less deformed than those on the lower surfaces.

Egg laying by the first summer brood females on the leaves starts in early June. On the average, each female lays about 12 eggs. In 1944, hatching was first observed on June 17, and in 1945, on June 19. These dates were previous to the completion of hatching of eggs of the overwintered scales. By mid-July of 1945 over half of the eggs under female scales on the leaves had hatched.

Male crawlers from eggs produced by the first summer brood females on the leaves tend to settle uniformly on the upper and lower leaf surfaces. In late summer, female crawlers migrate to the stems, and settle for the most part on the current season's wood. In mid-August, the rise in number of scales per twig is rapid until early October. Nearly all the scales settling on the stems develop into females which overwinter. Mating takes place in the fall. From July through the autumn months a large proportion of the live scale on the lower leaf surfaces is composed of females which are laying eggs. An overwintering, a first summer, and a partial second summer brood are indicated.

The overwintering females on the twig wood are so different in size and body characters that they have been thought to be a different species from the fig scale infesting the leaves in the summer. The former has been named *Lepidosaphes ficus* and the latter *L. ficifoliae*. The designation of two distinct species does not agree with the observations made in the field, however. Egg transfer experiments showed that *ficifoliae*-form females arise from eggs laid by the *ficus* form and that *ficus*-form females arise from eggs laid by the *ficifoliae*-form females. The result of these experiments confirms Lupo's work (1943). During the summer all of the female scales on the leaves were typical *ficifoliae* form. In October, a few *ficus*-form females were found on the leaves. These were generally easily distinguished by their shape and color. They were rarely found to be laying eggs.

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GENETICS AND DEVELOPMENT OF NINE MALE-STERILE TOMATO MUTANTS¹

CHARLES M. RICK²

SUMMARY

A search was conducted in commercial plantings of the San Marzano tomato for male-sterile mutants, potentially useful in producing F_1 hybrid seed and in cross-breeding. Among 150 unfruitful plants, 12 were found to be genetically male-sterile. Breeding results indicate that the male sterility of each mutant is determined by a single recessive gene. One gene— ms_5 —was recovered four times. Eight other nonallelic genes— ms_6 to ms_{18} —were demonstrated. No mutants for female sterility or cytoplasmic male sterility were found.

In the mutant ms_5 , 3 to 5 per cent of the microsporangia produced functional pollen; by using this pollen in self-pollination, pure-breeding male-sterile populations of ms_5 were obtained. No functional pollen was produced by other mutants.

The growth of anthers and, to a lesser extent, petals is suppressed to different degrees in the different mutants. The male-sterile segregates in some mutants can be identified macroscopically by the size and form of the floral parts, and in all of the mutants by the anther contents. Anthers are reduced most and breakdown of microsporogenesis occurs earliest in the four mutants whose petal length is significantly diminished. No effects of the genes were observed in other parts of the plant.

The genes for male sterility exert their most profound effects on the pollen mother-cells and early microsporogenesis. The mutants differ in time and rate of breakdown. The behavior of chromosomes is normal until the time of breakdown and does not seem to account for failure of pollen development.

Each gene affects tapetal development differently; in most mutants the breakdown is delayed, but in a few it occurs at the normal time or earlier. Pollen production is disrupted earliest in those mutants in which tapetal degeneration is delayed. Breakdown of microsporogenesis in ms_5 involves an erratic orientation of sporogenous tissue, the latter usurping the locus of the outer tapetum and wall tissue.

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TABLE 1

SUMMARY OF SEGREGATIONS OBTAINED FROM CROSSES MADE TO TEST IDENTITY OF THE MALE-STERILE MUTANTS

Plant and no.	1-1, <i>mas</i>	15-31, <i>mas</i>	16-44, <i>mas</i>	2-39, <i>mas</i>	2-52, <i>mas</i>	2-107, <i>mas</i>	2-124, <i>mas</i>	2-44, <i>mas</i>	2-89, <i>mas</i>	2-98, <i>mas</i>	2-121, <i>mas</i>	2-132, <i>mas</i>	2-152, <i>mas</i>	2-161, <i>mas</i>	2-165, <i>mas</i>
1-1, <i>mas</i>	23F†	27F	15F	10F 11F	10F 11F	12F	12F 12F	10F	9F 12F	11F	11F 12F	11F 12F	11F 12F	15F	9F
16-31, <i>mas</i>		9F	10F 12F	11F	11F	12F	12F 12F	13F 12F	10F 12F	11F	11F 12F	11F 12F	12F	12F	12F
16-44, <i>mas</i>			12F 12F	12F	12F	11F	13F 7F	12F 11F	12F 4F	9F 10F	11F 12F	11F 10F	12F	12F 10F	11F
2-39, <i>mas</i>				4F, 4S 12S*	10S*		6F, 6S 6F, 4S	9F 12F	9F 12F, 8S 4F, 8S	12F	11F 11F	11F 11F	5F 9F 11F, 1S	11F	12F
2-52, <i>mas</i>					6F, 8S 13F, 1S		4F, 8S 7F, 8S	11F	12F 12F	11F	14F 12F	10F 11F	12F 12F	12F 6F	13F
2-107, <i>mas</i>							12S* 12S*	17F	11F	12F 6F	8F 12F	11F 12F	12F 12F	10F	6F
2-124, <i>mas</i>								12F 12F	12F 8F	11F 12F	12F 10F	12F 12F	12F 12F		11F
2-44, <i>mas</i>									12F 11F	12F	12F 8F	12F 12F	12F 8F	10F	11F
2-89, <i>mas</i>										10F 10F	12F 10F	12F 12F	12F 12F	11F	11F
2-98, <i>mas</i>											11F 10F	10F 12F	14F 10F	10F	11F
2-121, <i>mas</i>												9F 10F	11F 12F	11F 13F	12F
2-132, <i>mas</i>													4F 5F	9F 14F	8F 9F
2-152, <i>mas</i>														8F	9F
2-161, <i>mas</i>															8F

† F signifies a fertile plant; S, a sterile one. Thus, 23F indicates 23 fertile plants.

* These families, consisting exclusively of sterile plants, were obtained by intercrossing plants of the *mas-mas* genotype, advantage being taken of the occasional fertile pollen produced by this homozygote.

Each cross consisted of a transfer of pollen from the heterozygote of one mutant to the homozygous recessive form of another, the operation being facilitated by the male-sterile condition of the latter, which obviated emasculation. If the genes in both parents were allelic, this would amount to a back-cross, and a segregation of approximately 50 per cent fertile and 50 per cent male-sterile plants would be expected. If the genes were not allelic, only fertile plants would appear. According to the binomial distribution in back-cross expectation, there is only one chance in $2^8 = 256$ that all individuals in a population of 8 would be fertile if the genes were allelic. Every effort was therefore made to secure test families of 8 or more plants in the 105 possible combinations of the 15 mutants.

The plants of these test families were grown and the phenotypes determined according to the methods outlined in the following section.

The segregations observed are presented in table 1. Duplicate and triplicate entries in any space usually indicate that direct and reciprocal crosses were tested, although in a few cases several crosses of the same direction were tested. Since male-sterile offspring appeared in all families of each of the six possible combinations of 2-39, 2-52, 2-107, and 2-124, these four mutants must be allelic. The genes in each are probably identical because no differences were observed in their effects. The symbol ms_s is therefore applied to each of these mutants.

While these test crosses were being studied, it was discovered that male-sterile segregates of ms_s occasionally produce a small quantity of functional pollen; consequently (as elaborated in the section on natural cross-pollination) they are subject to a low level of self-pollination. This condition was utilized to advantage by applying pollen of homozygous recessives instead of heterozygotes in subsequent test crosses. In such crosses allelism would be indicated by 100 per cent male-sterile progenies; while nonallelic combinations would be indicated by 100 per cent fertile progenies, and an F_1 consisting of a single plant would therefore provide an adequate test. Several families of 100 per cent male-sterile plants were actually obtained in this fashion (table 1). Production of viable pollen has not been observed at any time in any of the other male-sterile mutants.

In the light of these facts, several apparent discrepancies in table 1 can be explained. Three families in other test crosses (2-39 \times 2-89, 2-39 \times 2-152, and 2-52 \times 2-132) also segregated for male sterility. Since the male sterility of 2-39, 2-52, 2-107, and 2-124 is determined by the same gene, all crosses between these four mutants and any other single mutant constitute duplicate tests of a single combination. Thus the three segregating families prove to be exceptional because each one disagrees with seven or eight other families consisting exclusively of fertile plants that test the same combination. In other words, if these segregations indicate that 2-89, 2-152, and 2-132 are allelic with ms_s , 23 other families should also segregate, but none of them does. Since each exceptional family is generated by a cross between ms_s as the pistillate parent and some other mutant as the staminate parent, the male-sterile plants in these three exceptional families are construed as the products of self-pollination of the ms_s parent. According to this explanation, the proportion of male-sterile plants in the exceptional families might be expected

TABLE 2
SEGREGATIONS IN F₂ AND BACKCROSS GENERATIONS

[illegible]

to be erratic and not to conform necessarily with a 1:1 ratio. This expectation is realized, for the proportions in the three families are 67, 25, and 8 per cent, the latter being a highly significant deviation from the 1:1 ratio.

The information that is apparently lacking in the absence of any family of the combination 2-124 \times 2-161 and is apparently deficient in the family of only 6 individuals in the combination 2-107 \times 2-165 is supplied by the test crosses between the second parent of these crosses and the other representatives of *ms₅*.

Since the male sterility of each of the other mutants is determined by a different gene, the symbols *ms₆* to *ms₁₃* are applied to them in order of the sequence of their discovery. Of the 12 original male-sterile plants discovered in this survey, therefore, nine are determined by different genes, the other three proving to be duplicates of one of the nine genes.

INHERITANCE

Backcross and F_2 populations of the nine mutants were grown in 1945, 1946, and 1947. The population referred to here as a family consists only of the plants grown from seeds that were formed in a single fruit. By separating families in this manner it was possible to limit errors in pollination to the seeds of single fruits, and in this way avoid contamination of an entire seed lot of any given cross or selfing. Single-fruit families of the variety San Marzano consist of 15 to 60 plants.

The families were sown in nursery pots, from which the seedlings were transplanted to 2 \times 2 inch spacing in flats; from the flats the plants were shifted to the permanent planting in the field, where they were spaced 1 foot apart in rows spaced 6 feet apart. This field spacing was sufficient for the plant to grow vigorously for the first 2 months, so that flower types could be identified reliably in the first three or four inflorescences.

The phenotype of each plant in these families and also in the test progenies of the preceding section was determined by examination of anther contents smeared in acetocarmine. This microscopic test was not necessary for accurate detection of any of the male-sterile mutants; nevertheless, it was used in order to have absolute determination of all plants and also to observe how much different plants of the same mutant might vary in the contents of their anthers. Many of the same families were examined later in the season to see which plants had set fruit. With very few exceptions, unfruitfulness was observed only in those plants that had been identified earlier as male-sterile by the nature of their anther contents.

The observed segregations were compared with the expected values by means of the chi-square test, and the share of the chi-square contributed by heterogeneity between families of the F_2 , and the backcross of each mutant was computed. These data are summarized in table 2. According to these calculations, heterogeneity at the 5 per cent level of significance is indicated for only one group of data—namely, F_2 families of the *ms₅* mutant. No significance can be legitimately attached to this departure because, by its very definition, such a deviation might be expected to occur on the average once in every 20 groups. This single deviation appearing in 17 groups would therefore not be exceptional.

Recessive monogenic inheritance is indicated for every mutant tested; the observed segregations conform well with expected values. Deficiencies in the numbers of male-sterile plants that are significant at the 5 per cent level exist in 2 of the 17 groups of data. The significance of 2 such deviations might be questioned in the same light as the evidence for heterogeneity. More noteworthy are the facts that both these deviations are deficiencies of male-sterile plants and that the latter are also deficient in the totals of all F_2 and backcross individuals. The departure is not significant in the F_2 totals, but does exceed the number corresponding to the 5 per cent level of significance for the backcross totals.

Only in the case of ms_5 do both F_2 and backcross progenies deviate in the same direction. Although the deficiency of recessives is statistically significant only in the backcross, the consistent deviation is suggestive. Since the great variability in size and contents of anthers (elaborated in a subsequent section) might have led to misclassification of recessives as dominants, this mutant might merit additional intensive genetic study.

These results correspond with the deficiency of recessive homozygotes reported for a great many Mendelian characters. It can be concluded, therefore, that male sterility in each of these nine mutants is conditioned by a single recessive gene.

MACROSCOPIC CHARACTERISTICS

For the sake of convenience, the diploid plants that exhibit normal fertility, whether heterozygous for ms genes or homozygous for their dominant alleles, are designated here the "normal type" to contrast them with the mutant types.

Differences between the male-sterile and normal-type segregates of each of the nine mutants have been observed only in the flowers. Macroscopic differences in vegetative characters, by means of which the two types could be identified before plants flower, were eagerly sought, especially because they might be useful in large-scale production of male-sterile plants; but none were found.

Macroscopic differences in flowers, however, are readily seen in most of the mutants. As expected, these differences chiefly concern the anthers. Representative samples of anther tubes of the normal type and male-sterile segregates of each mutant were photographed at low-power magnification and are reproduced in plate 1. The range of effects on anther form are shown to vary from the extremely reduced anthers of ms_5 and ms_{10} to the least modified ones of ms_7 , ms_8 , and ms_{13} . The modification of anther form wrought by each gene appears consistently in the great majority of the flowers, except those conditioned by the gene ms_5 .

The product of length and diameter of anther tube provides a measure of anther size that is useful for comparing mutants. The means of such products are included in table 3. According to these figures, all mutant anthers are smaller than normal. Though this difference is statistically significant in all comparisons with the normal type, the distributions in the case of ms_7 and ms_8 overlap that of the normal; therefore, even size determinations based on measurements would not always provide a reliable separation of fertile and sterile segregates.

Certain of the male-sterile mutants differed from the normal in anther color (table 3). The departure from the normal orange-yellow color is sufficient in some of the mutants (ms_5 , ms_{10} , ms_{11} , ms_{13}) to be as useful in distinguishing sterile segregates from fertile ones as the differences in size and form. The deviation is always toward a paler color, varying from a tone only slightly lighter than normal, as in ms_{13} , to a decidedly lighter tone—a pale lemon yellow—as in ms_{10} . Aside from these differences, a very noticeable brownish discoloration, concentrated in the region of the dehiscence slit is frequently observed in anthers of ms_3 . Slight as the difference is in ms_{13} , it is an aid in identifying sterile segregates.

TABLE 3
SUMMARY OF MACROSCOPIC CHARACTERS OF THE FLOWER

Mutant number	Petal length			Significance of difference in petal lengths		Anther tube length \times diameter	Anther color
	Mean of fertile segregates	Mean of sterile segregates	Difference	<i>t</i>	<i>P</i>		
ms_5	mm 12.96	mm 11.40	mm -1.56	-4.69	<0.01	2.33	Lemon-yellow; frequent brownish discoloration
ms_6	11.39	10.94	-0.45	-1.01	0.3-0.4	3.19	Lemon-yellow
ms_7	12.84	13.00	0.16	0.42	0.6-0.7	4.36	Orange-yellow
ms_8	12.30	10.52	-1.78	-4.98	<0.01	2.79	Golden-yellow
ms_9	12.29	11.47	-0.82	-1.34	0.3-0.4	4.13	Orange-yellow
ms_{10}	13.07	11.24	-1.83	-2.95	<0.01	2.56	Greenish-yellow
ms_{11}	12.29	12.31	0.02	0.05	0.9	2.93	Lemon-yellow
ms_{12}	12.35	10.50	-1.85	-4.07	<0.01	3.14	Orange-yellow
ms_{13}	11.88	11.63	-0.25	-0.46	0.6-0.7	3.83	Golden-yellow
Normal type	0.00	4.87	Orange-yellow

No matter how similar to the normal type the anthers of certain male-sterile mutants may appear, it is always possible to differentiate them from normal segregates by a macroscopic examination of the contents of their anther locules. By prodding the anthers apart with a flattened instrument, one can readily determine in the field whether or not pollen is produced. If aborted pollen is produced, as in ms_{13} and other mutants, it can be distinguished from functional pollen by its much smaller mass. Although this method is more tedious than a casual examination of anthers, it is a macroscopic method that is always reliable for detecting the male-sterile segregates of each of the nine mutants.

The anther tubes in plate 1, C, represent the extremes of the normal range of variation for ms_5 . Flowers exhibiting various degrees of modification of the anthers within this range appear on the same plant at the same period, this range being typical of the ms_5 gene and apparently independent of plant or seasonal differences.

Examination of a large number of flowers of this mutant has shown that the small amount of functional pollen it produces is generated by the anthers that most closely approach the normal type. Male-sterile mutants that produce limited quantities of functional pollen are also known in *Zea* (Beadle, 1932).

Reduction of corolla size is another feature associated with the effects on anthers in some of the male-sterile mutants. For comparison, the petal length was measured in a single flower from each of 15 to 25 plants of each of the two segregates—fertile and male-sterile—of backcross populations of the nine mutants. Such measurements of the sterile and fertile segregates of the same population are required to reveal the differences in corolla size, which are much less extreme than those in anther size. Means were computed and differences compared by means of Fisher's (1946) *t* test (table 3). Highly

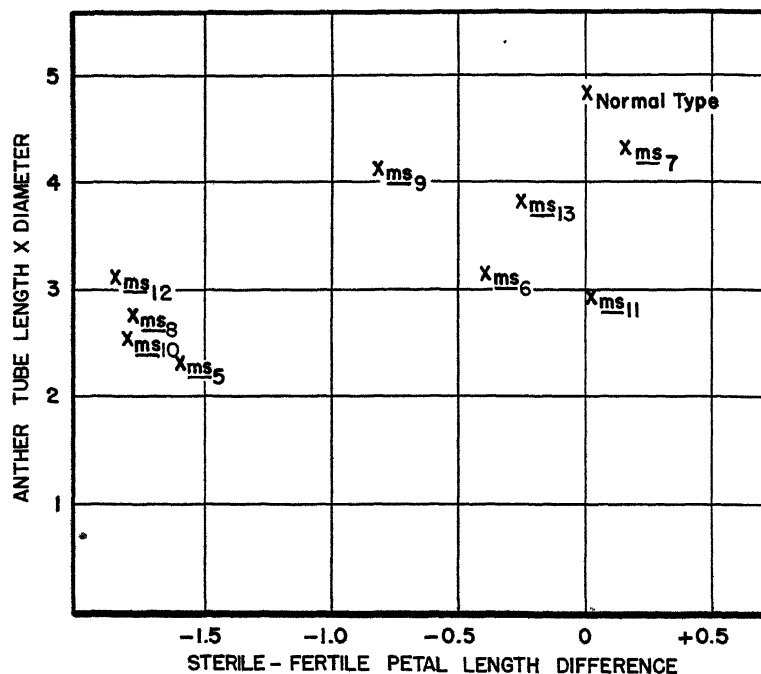


Fig. 1. Scatter diagram of the nine male-sterile mutants and the normal type, showing the relations between size of anther tube and length of petal.

significant decreases in petal length are characteristic of the male-sterile segregates of *ms*₅, *ms*₈, *ms*₁₀, and *ms*₁₂. In respect to the difference in petal lengths, these four mutants are sharply separated from the other five, in which any differences fall far short of significance. The gap between these two groups is illustrated in figure 1, in which this difference between petal lengths is plotted against the anther-tube measurements for each of the nine mutants and the normal type. The anther-tube measurements for the four mutants having significantly shorter petals in the male-sterile segregates are also below those for the remaining group of mutants except *ms*₁₁ (table 3 and fig. 1). Within either group, however, the two variables do not seem to be correlated. This rather unusual segregation of the mutants also corresponds with the time of internal breakdown of the anthers and will be mentioned later.

Though four of the genes appreciably reduce petal lengths, the frequency distributions of fertile and sterile segregates overlap considerably in all cases.

In other words, the effect on petal length is not nearly so drastic as the effects on anther length or girth, or the product of the two, for which measurements nonoverlapping distributions are obtained for all except two mutants.

DEVELOPMENT OF ANTHERS

The development of anthers of each of the mutants was studied and compared with similar stages of the normal type. The preparations consisted of fixed and sectioned anthers and others that were smeared directly in aceto-carmin. Useful information was provided by both techniques; but the smears were more helpful as an aid to the study of pollen mother-cells and male gametophytes; while the sectioned preparations were necessary for a study of the nonsporogenous tissues and their temporal and spatial relations with the sporogenous tissue.

In a preliminary study of the preparations it soon became apparent that the action of the nine genes is essentially limited to the pollen mother-cells, the subsequently developed male gametophytes, and adjacent tissues of the anther. As mentioned earlier, some of the genes tend to reduce the size of corollas and to have a still more drastic effect on size of anther, but these differences are not accompanied by striking internal changes except in the aforementioned tissues. Since such other tissues of the anther as the sterile parenchyma, conducting tissue, and the endothecium show no deviation, save possibly slight reductions in cell size, no reference will be made to their development except those aspects that might bear on the gene effects described.

This account is also limited in respect to time of development. In stages preceding the final mitoses that delimit the pollen mother-cells, no deviation from the normal type was observed in most of the mutants. Attention will therefore be directed mostly to the development from the time of differentiation of the pollen mother-cells until anthesis.

It was essential to establish the developmental age of the anthers before the mutants could be compared with the normal type. While size of anthers does provide a general approximation, it is not reliable for precise timing because anthers are considerably reduced in many of the mutants. The developmental stage of certain unaffected tissues of the flower provides a more exact measure of the relative age of the anthers. One example is the developmental sequence in petals: in transverse sections through the middle of the flower bud, petal primordia are well separated in early stages, approach each other later, and eventually meet and buckle inward in the dorsal groove of each anther. While the petals provide an adequate way to measure stages in early development, the length of epidermal hairs, which appear on the anthers in later stages (Rick, 1947), is more reliable for intermediate stages of this period. Timing of the final stages of development including anthesis of the flower can be followed accurately in the differentiation of those endothelial cells that regulate the opening of the dehiscence slit. In the final stages these cells elongate radially and they retain an active condition longer than any other sterile cells of the anther. A complete delineation of all these sequences would not be warranted here. It is sufficient to say that by examining these tissues one can classify anthers without difficulty into numerous stages of development between the early differentiation of pollen mother-cells and anthesis.

TABLE 4
SUMMARY OF MICROSPOROGENESIS AND DEVELOPMENT OF THE MALE GAMETOPHYTE AND OF THE TAPETUM

Mutant no.	Microsporogenesis and development of the male gametophyte			Development of the tapetum			Appearance of microsporangial cavity at anthesis*
	Rate of development	Stage of breakdown	Appearance at anthesis	Time of breakdown	Size of cells at breakdown	Appearance at anthesis	
Normal type	Normal	Mature pollen	Meiosis	10-15 × PMC	Faint vestiges	Well expanded
<i>ms1</i>	Slightly delayed	Mostly I ₁ prophase	No remains	Delayed	5-8 × PMC	Compressed dark layer	No cavity
<i>ms2</i>	Variously delayed	4-nucleate PMC or tetrads	Indistinguishable	Meiosis	Same size as PMC	Compressed dark layer	Slightly expanded
<i>ms7</i>	Variously delayed	I ₁ prophase to mid-microspore	Aborted pollen mostly in tetrads	Meiosis	10-12 × PMC	Faint vestiges	Well expanded
<i>ms8</i>	Moderately delayed	I ₁ prophase to tetrads	Amorphous dark mass	Greatly delayed	5-8 × PMC	Intact cell walls	Moderately expanded
<i>ms9</i>	Normal	Early microspore	Indistinguishable	Slightly delayed	10-15 × PMC	More persistent than normal	Well expanded
<i>ms10</i>	Moderately delayed	Mostly I ₁ prophase	Indistinguishable	Greatly delayed	Same size as PMC	Intact cell walls	No cavity
<i>ms11</i>	Moderately delayed	Tetrad and early microspore	Aborted pollen free or in tetrads	Accelerated	5-8 × PMC	More persistent than normal	Moderately expanded
<i>ms12</i>	Moderately delayed	I ₁ prophase to tetrads	Indistinguishable	Delayed	5-8 × PMC	More persistent than normal	Slightly expanded
<i>ms13</i>	Normal	Late microspore	Aborted pollen	Variable	1-10 × PMC	More persistent than normal	Well expanded

* Appearance in transverse section.

The substance of this section—that is, a comparison of the development of anthers of the nine mutants and the normal type—is presented in outline form in table 4.

The observations can be presented most efficiently by discussing first the development of the normal-type structures and by treating only those aspects of the mutants that depart from the normal pattern.

Microsporogenesis and Development of the Male Gametophyte in the Normal Type. Gametogenesis in the tomato, including the early development of floral organs and derivation of the sporogenous layers, has been described in detail by Smith (1935). Sporogenous tissue is differentiated very early in the development of the anther. As seen in a transverse section of the anther, it appears as a crescentic mass, several cells in thickness. The pollen-mother-cells are readily distinguished from other cells by their very dark-staining cytoplasm. Their nuclei gradually enlarge until, in prophase of the first meiotic division, they nearly equal the volume of the cytoplasm of the cell. The two meiotic divisions proceed very rapidly, giving rise to a four-nucleate pollen mother-cell. The four-nucleate stage and the subsequent one in which four microspores are delimited by cell walls persist for much longer periods than the meiotic divisions. After the uninucleate microspores are released from the old pollen-mother-cell wall, they quickly assume a spherical shape and enlarge until each finally reaches a volume comparable to that of the original pollen mother-cells.

The microspores become refractory for study of nuclear behavior early in this final period because their cytoplasm changes to a dense, granular condition. According to Smith a single mitosis occurs during this interval, the mature pollen grain containing a generative and a tube nucleus. The sequence of development giving rise to mature pollen grains in tomatoes, accordingly, is typical of many angiosperms.

Development of the Tapetum and Other Tissues in the Normal Type. In the tomato the tapetum is a single layer of cells that lines the microsporangial cavity and serves as a nutritive layer for the developing pollen mother-cells and subsequently developed microspores. In keeping with its nutritive function the tapetum develops cataclysmically.

The first evidence of the tapetum is seen in the single layer of cells on the adaxial side of the sporogenous layer. At the time the cells of the latter layer first appear darker, the former enlarge and become decidedly more vacuolate than the other adjacent cells. Somewhat later, sterile cells on the abaxial side differentiate in the same manner, until the sporogenous layer is completely enveloped by this layer of strikingly contrasting appearance. The nuclei of the tapetal cells enlarge rapidly and, in many instances, divide to produce permanently binucleate cells in early development. The cells enlarge so rapidly that, by the time the pollen mother-cells have reached mid-prophase of the first meiotic division, they have attained a volume ten to fifteen times that of the latter and have greatly distended the microsporangia. Fixation of the tapetum is most difficult at this time of maximum expansion.

Degeneration starts at various times, but is generally evident by the time of the first and second meiotic metaphases in the pollen mother-cells. The cells collapse to a dark-staining layer lining the greatly enlarged microsporangial

cavity. Degeneration continues until, at anthesis, nothing of the tapetal cells remains but a threadlike vestige in the periphery of the cavity (plate 2, *A*).

Since it is concerned in the development of one of the male-sterile mutants, the wall tissue of the anther should be mentioned here. At the time the sporogenous tissue is first differentiated, it is protected on the abaxial side by a layer three or more cells in thickness. Cell divisions continue until this layer is at least five cells thick. These cells are parenchymatous and are greatly flattened radially. As mentioned previously, the endothecium—the outermost layer of the anther wall nearest the junction of the two microsporangia of an anther lobe—develops in a special manner so as to serve as the disjunctive layer.

Development of Anthers in the Mutant Types. Discussion of development of *ms_g* is withheld until the end of this section because its characteristics are exceptional.

ms_g. Microsporogenesis stops in the four-nucleate stage of the pollen mother-cells or in the immediately subsequent tetrad stage. Development is normal up to meiotic prophase; after that meiosis is delayed to varying degrees. In some cases meiosis is still in progress in anthers in which large microspores would be present in the normal type. In spite of this variability, meiosis seems to proceed normally and to be completed in all pollen mother-cells. The tetrads or four-nucleate pollen mother-cells degenerate rapidly, and by anthesis nothing remains of them save an amorphous dark-staining layer (plate 2, *C*).

Tapetal cells of this mutant differentiate normally in early development, but never enlarge to any greater extent than the pollen mother-cells. In most preparations they begin to disintegrate at the same time as in the normal type despite their exceedingly subnormal size, although occasionally this disintegration is slightly delayed. Collapse and disintegration quickly follow, and the remains of this tissue may or may not line the periphery of the slightly enlarged microsporangial cavity. At anthesis the dark-staining mass of the tapetum is coalesced with the remains of the pollen mother-cells (plate 2, *C*).

ms₇. Gametogenesis is interrupted at various stages in this mutant. Abortion has been observed at all stages from prophase of meiosis to intermediate stages of microspore development. Cells that are affected in early stages are either resorbed or so greatly modified that they cannot be recognized as such • at anthesis. Aborted microspores still adherent in tetrads are seen in fully half the anther smears of this mutant, and microspores aborted in later development also persist to anthesis in recognizable form. Though the stage of degeneration is variable in this mutant, the time of breakdown appears to be quite precise; in other words, the rate of microsporogenesis varies considerably from one cell to another, but all cells of an anther degenerate at approximately the same time, some still being in various stages of meiosis, others having reached the microspore stage. Many of the aborted microspores are retained in tetrads, even until anthesis (plate 2, *D*).

The tapetum develops normally in respect to the time of the different changes. The cells do not reach quite so large a size as they do in the normal type. The greatly enlarged locular cavities at anthesis attest to the expansion of the tapetal cells of this mutant. Only a faint vestige of them remains at anthesis (plate 2, *D*).

ms₈. Degeneration of microsporogenesis somewhat resembles that of *ms₇*. Breakdown may occur in various stages of meiosis from prophase to the formation of tetrads. It differs in timing, however, for all the meiotic figures appear somewhat later than they do in the normal type. The remains of pollen mother-cells appear at anthesis only as a compressed dark-staining mass (plate 2, *E*).

The development of tapetal cells deviates from the normal type in the respect that it is delayed. Early differentiation of the cells appears normal, but they enlarge at a slower rate and finally reach a maximum size, only half that of tapetal cells in the normal type, much later than they do in normal material. The delay in development is also manifested in their slow disintegration, together with the fact that, at anthesis, most of the walls of the tapetal cells are still intact (plate 2, *E*). In this last respect *ms₈* differs strikingly from *ms₆*.

ms₉. Breakdown occurs with striking precision in the early microspore stage. All aspects of earlier development seem normal; meiosis is not delayed, and it results in the formation of normal-appearing microspores, which are regularly released from their tetrad groupings. These microspores do not enlarge, however, but gradually lose their contents, until, at the time that they would normally be considered midway in their development into pollen grains, they have entirely lost their contents. At anthesis the microspores are so completely degenerated that they are scarcely recognizable (plate 2, *F*). Even though microspores mostly degenerate later in *ms₉* than in *ms₇*, they are more quickly resorbed.

Aside from a slight delay, the tapetum develops in *ms₉* as it does in the normal type, the cells enlarging to nearly the same extent. The delay is manifested at anthesis by cell remains that are more persistent than in the normal type (plate 2, *F*).

ms₁₀. Meiosis is initiated somewhat later than in the normal type. Degeneration occurs in early prophase of meiosis in all save about 15 per cent of the buds, in which a few cells are able to reach later stages, even as late as tetrads. Rapid degeneration follows, reducing the clumped pollen mother-cells to unrecognizable masses (plate 3, *A*).

In some respects the sequence of tapetal development in *ms₁₀* resembles that of *ms₈*. Early differentiation of the cells is normal, but enlargement is slow and greatly delayed. The tissue, in fact, never seems to degenerate, the cells appearing intact at anthesis, although generally lacking contents. Since the tapetum never shrinks, no cavity appears in the microsporangia at anthesis (plate 3, *A*).

ms₁₁. The pattern of breakdown is similar in some respects to that of some of the mutants already discussed, but in every comparison it differs in certain characteristics. Early development, including meiotic prophase, is normal. Later stages of meiosis appear normal, but they are considerably delayed. Pollen mother-cells rarely disintegrate before the tetrad stage. About half the microspores abort while in tetrads; the remainder collapse after release. Whenever they break down, the microspores remain juvenile in size and form, slowly lose their cytoplasmic contents, and appear at anthesis as empty shells, either free or attached in tetrads (plate 3, *B*).

Tapetal development in ms_{11} is anomalous inasmuch as disintegration begins slightly earlier than in the normal type, yet proceeds at a slower rate. In the earliest stages the cells increase in size at the normal rate, but they cease to do so long before they reach the great expansion attained in the normal type. Their subsequent contraction leaves moderately large cavities in the microsporangia. As in the case of ms_6 , the cell walls are more or less intact at anthesis (plate 3, *B*).

ms_{12} . The interruption of microsporogenesis is strikingly like that of ms_8 . Meiosis is often considerably delayed, and cells may disintegrate at any stage from prophase until the formation of tetrads, although most collapse in prophase. The remains of the pollen mother-cells aggregate with the tapetum to form an irregular dark-staining layer at anthesis (plate 3, *C*).

Tapetal cells develop somewhat more slowly than normal in ms_{12} . After reaching a volume of about half that of normal material they begin their degeneration at a later stage than normal. They deviate from the normal also in shifting toward the center of the cavity instead of toward the periphery. This behavior may account for the smallness of the locular cavity. The remains of the tapetum are more persistent in ms_{12} than they are in the normal type (plate 3, *C*).

ms_{13} . Male gametophytes reach a later stage of development in ms_{13} than they do in any other mutant. All development is normal until microspores reach a fairly large size. Here their enlargement is arrested and they rapidly lose their cytoplasmic contents. They are easily recognized at anthesis as relatively large, but shriveled and empty microspores (plate 3, *D*).

The tapetum develops in a more variable manner in ms_{13} than in any other mutant. Some cells, for instance, may degenerate in very early stages before expanding to any extent; others may expand considerably, but never to the size realized in the normal type. The cells disintegrate, but not quite so completely as in the normal type (plate 3, *D*).

ms_5 . The description of development of ms_5 is delayed to this point because, in contrast to the other mutants, its microsporangia may follow different patterns of development. Only 3 to 5 per cent develop in the normal fashion and generate normal pollen. Most of the remainder deviate in a single general pattern, although a small percentage develop in an intermediate fashion.

From very early developmental stages of the anther, most microsporangia of this mutant are abnormal. As soon as the sporogenous cells assume their densely cytoplasmic character, it is observed that *they consist not only of a C-shaped mass of two cells' thickness, but also of all abaxial cells up to the epidermal layer* (plate 3, *F*). This anomalous condition persists through later stages and seems to be responsible for the final breakdown in pollen production. In 90 per cent of the microsporangia that deviate in this manner, pollen mother-cells degenerate in prophase of meiosis, the remainder collapsing somewhat later. Though the cells may collapse without any delay, meiosis is usually retarded.

In the deviating microsporangia, an abaxial tapetum apparently never appears where the sporogenous cells extend to the epidermis (plate 3, *F*). The correspondence of tissues in early and late stages in the preparations gives this impression, yet it would be difficult to prove that outer cells of the

PLATES

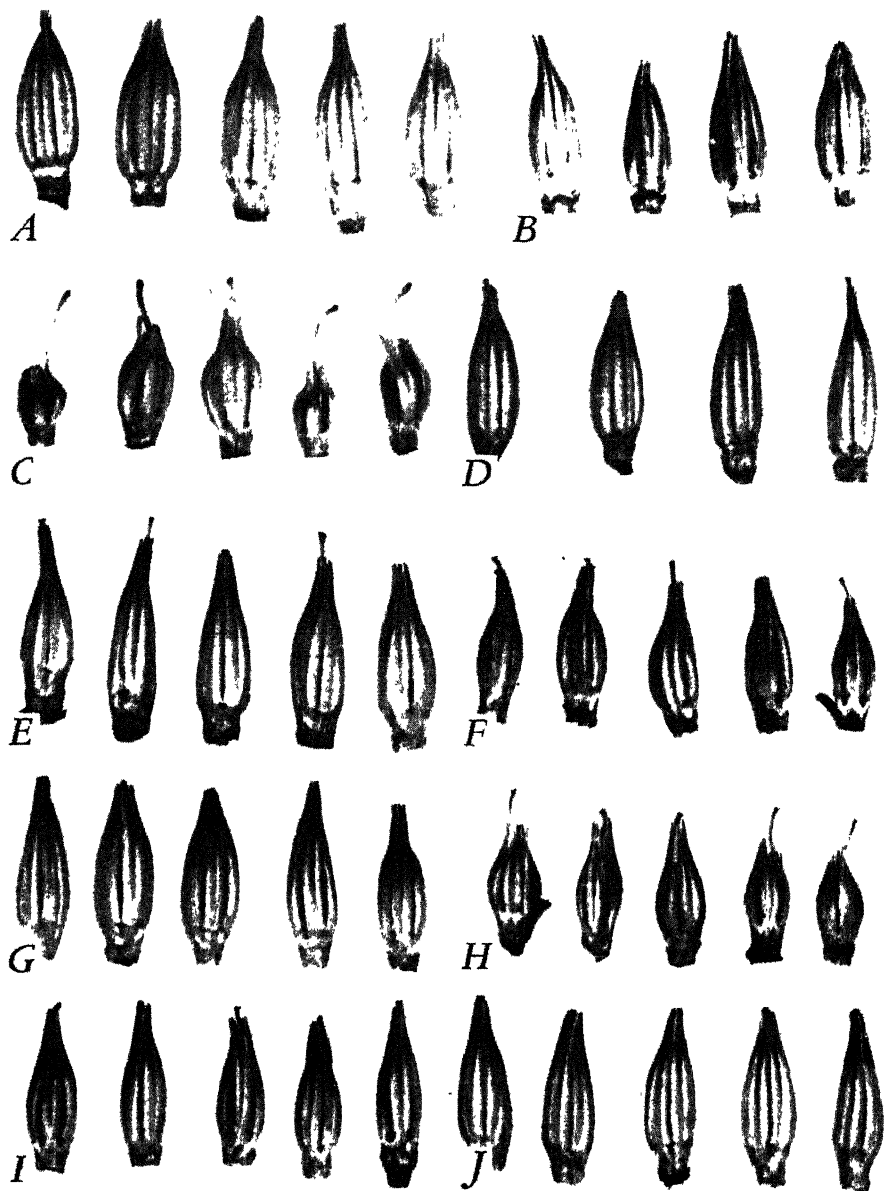


Plate 1. Anther tubes of the normal type and male-sterile mutants: A, normal type; B, ms_{11} ; C, ms_5 ; D, ms_6 ; E, ms_7 ; F, ms_8 ; G, ms_9 ; H, ms_{10} ; I, ms_{12} ; J, ms_{13} . (All $\times 3$.)



Plate 2. Transverse sections of anther lobes of the normal type and male-sterile mutants. All except *D* were collected just prior to anthesis; *D* represents a slightly earlier stage. *A*, normal type; *B*, *ms₆*, showing normal-type development in the upper microsporangium and the much more frequent degeneration in the lower microsporangium; *C*, *ms₆*; *D*, *ms₆*; *E*, *ms₆*; *F*, *ms₆*. (All $\times 100$.)

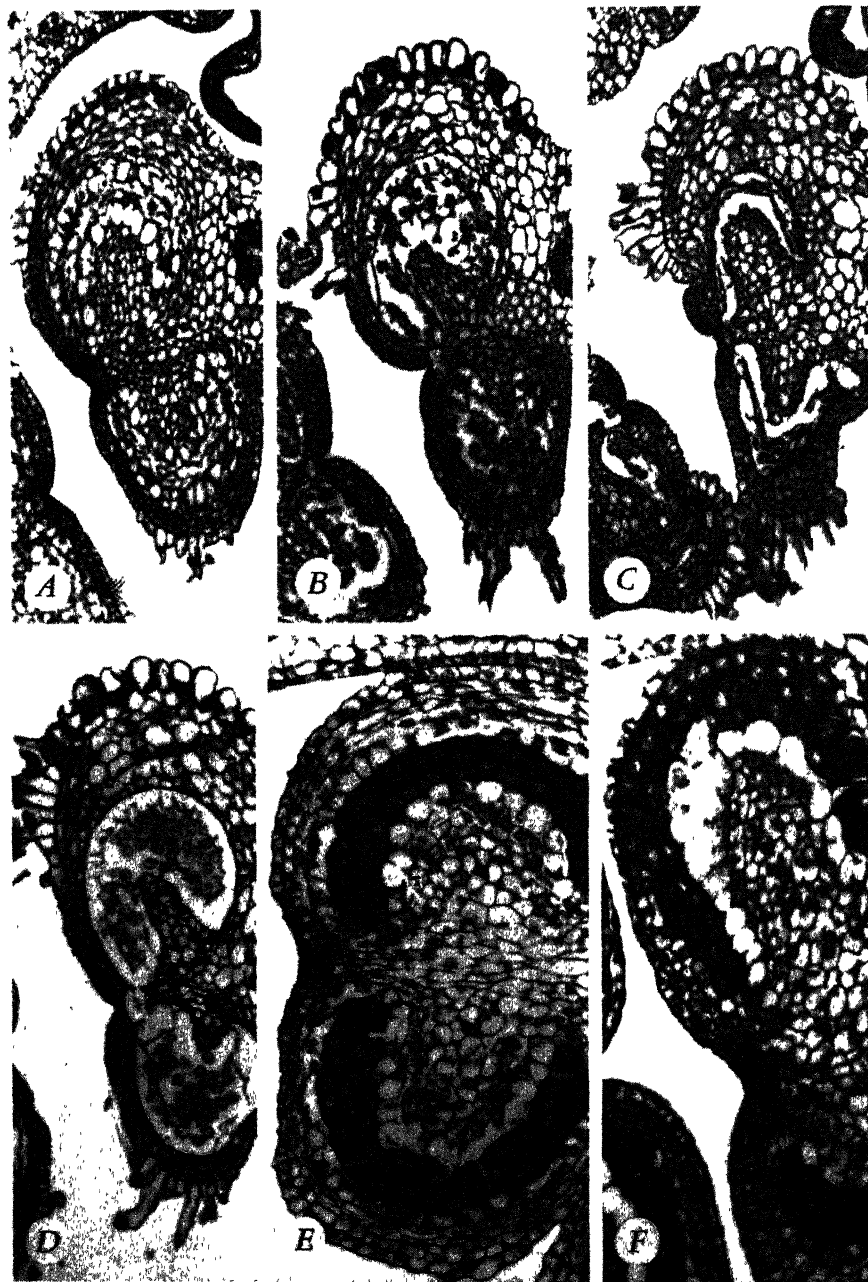


Plate 3. Transverse sections of anther lobes of male-sterile mutants. A-D were collected just prior to anthesis; E and F represent an early stage in the differentiation of pollen mother-cells. A, *ms₁₀*; B, *ms₁₁*; C, *ms₁₂*; D, *ms₁₃*; E, normal type, showing the location of the darkly stained pollen mother-cells; F, *ms₆*, showing aberrant location of pollen mother-cells in the upper microsporangium and normal location in the lower one. (A-D, $\times 100$; E and F, $\times 200$.)

dark-staining mass never develop tapetal properties. Tapetal cells develop continuously on the adaxial side and at either end of the mass of sporogenous cells on the abaxial side wherever sterile cells separate the sporogenous cells from the epidermis (plate 3, *F*). Tapetal cells that differentiate develop at a slower rate, reach a lower maximum volume, and collapse later than in the normal type. At anthesis they appear as part of the dark, compressed layer that includes remains of the pollen mother-cells, or occasional cells may retain their normal outlines (plate 2, *B*).

General Aspects of Anther Development. The considerations of anther development can be concluded with several statements of general significance:

1. *The breakdown of microsporogenesis or of the male gametophyte occurs differently in each of the nine mutants.* Cells may degenerate in different stages from early prophase of meiosis in the pollen mother-cells to relatively large microspores. Normal development of the early stages may proceed at the normal rate, or it may be delayed to varying degrees; furthermore, breakdown may differ in rate, even though it might start at the same time. The mutants ms_8 and ms_{12} resemble each other to the greatest extent in this respect, but even in this pair, in which the breakdown occurs in all stages of meiosis, the pollen mother-cells tend to degenerate earlier and with less variation in ms_8 than in ms_{12} ; furthermore, the pattern of tapetal development differs considerably in these two mutants.

2. *Until the time of breakdown, meiotic chromosomes behave normally.* In none of the mutants was there any evidence that abnormal chromosome behavior primarily causes the breakdown and consequent male sterility.

3. *Development of the tapetum is affected in all mutants.* The closest approach to normal development was observed in ms_7 , but, even in this mutant, tapetal cells do not seem to reach normal maximum size. The patterns of deviation in this development are almost as varied as those of microsporogenesis: the tapetum may degenerate sooner than it does in the normal type (ms_{11} , ms_{13}), at the same time (ms_7), or later to varying degrees (in the remaining mutants); the maximum size attained by tapetal cells varies greatly; and the cell remains persist to varying degrees at anthesis.

It is tempting to propose in the face of this evidence that the genes for male sterility act fundamentally upon the tapetum, and that, since the pollen mother-cells and microspores are nurtured by the tapetum, the effects on microsporogenesis and development of the microspore are secondary. On the basis of this hypothesis, the degree of deviation of tapetal development might be expected to agree with the degree of deviation of pollen production. These two effects are somewhat correlated, but the agreement is not striking—for instance, tapetal development is least modified in ms_7 , yet the breakdown in pollen production occurs earlier in this mutant than it does in ms_8 and ms_{13} , in which mutants tapetal development is more adversely affected.

The breakdown of the greatly enlarged tapetal cells is a feature of normal development; it is probably another manifestation of the transfer of nourishment from the tapetum to the pollen mother-cells and microspores in the same fashion that the embryo in many angiosperms develops at the expense of the endosperm. Hence a delayed breakdown of the tapetum might be construed as a more unfavorable influence on pollen production than one occurring at

the normal time or even a premature breakdown. Earlier breakdown of pollen production does occur in mutants in which the degeneration of the tapetum is delayed, and the earliest breakdown is associated with the most persistent tapetum (ms_5 , ms_{10}). But, even if a perfect correlation could be drawn between the two processes, the effects both on pollen production and on development of the tapetum might still conceivably be wrought by an earlier and more fundamental action of the gene.

In cytoplasmically male-sterile sugar beets, Artschwager (1947) found that tapetal cells may lose their cell boundaries to form a periplasmodium, which invades the anther cavity at the expense of the microspores. This abnormal development was not found in the noncytoplasmic type; neither does it occur in any of the tomato mutants of the present study.

4. *The genes conditioning male sterility act with remarkable precision.* According to the foregoing account, the degree of precision varies, but, even in instances of variable effects, the precision is evident. Microsporogenesis in ms_7 , for example, may be disrupted in any stage of meiosis, but it is limited to these stages; furthermore the full range of variation conditioned by the gene appears consistently in each flower bud. In respect to the development of normal pollen in certain microsporangia and interruptions at various stages in others, the mode of action of the ms_5 gene is less precise. When degeneration sets in during the microspore stage, the precision of gene effects is reflected in the uniformity of appearance of aborted microspores at anthesis. Thus, in ms_{13} , in which microspores break down at a relatively late stage, they exhibit remarkably little variation in size and afford a marked contrast with the extremely variable microspores produced by triploids, haploids, and asynaptic tomatoes.

5. *The breakdown in tapetal development or in pollen production is somewhat correlated with size and shape of mature anthers and with size of corolla.* The earliest degeneration in microsporogenesis and the most extreme deviation in tapetal development occur in ms_{10} , which also has the most deformed anthers; and the latest breakdown of development is associated with rather slight deformity of anthers in ms_9 and ms_{13} . No correlation exists, however, in the group of mutants that is intermediate with respect to anther form and time of degeneration. It is noteworthy also that the four mutants (ms_5 , ms_8 , ms_{10} , ms_{12}) whose petal lengths are significantly reduced are also uniformly characterized by earlier breakdown of microsporogenesis than the other mutants. No correlation is found, however, within the group of significantly lesser petal length or within the group of mutants whose petal growth is not markedly affected.

In view of the less specific effect, and, in some examples, seeming absence of effect on size and form of anthers and corolla, it seems likely that these are not the primary effects of the gene, upon which the breakdown of pollen production depends. Evidence supporting this view is contributed by the fact that other genes in the tomato can exert such more drastic effects on the corolla as a reduction in the number or size of petals or a complete suppression of the corolla, with only a moderate, or even no, effect on pollen production (unpublished). These morphological effects of the male-sterility genes are certainly correlated with the effects on pollen production, and they both

probably accrue from an earlier, more fundamental action of the gene, possibly on developmental hormones; but it appears very doubtful whether the breakdown of pollen production is a sequel to the general reduction in size of flower parts.

OVULE FERTILITY

Since the genes for male sterility exert such a pronounced effect on microsporogenesis and development of the male gametophyte, it is of interest to know what effect, if any, they have on the formation of ovules. From the start of this investigation it was known that each mutant was at least moderately fertile as a pistillate parent; otherwise, they would not have been recognized as male-sterile mutants. Yet a considerable abortion of ovules might easily have been overlooked in the preliminary observations.

TABLE 5
SUMMARY OF DATA ON OVULE FERTILITY

Mutant no.	Total ovules examined	Per cent ovules of			Total fruits examined	Mean number of seeds per fruit			
		Normal type	Substitution type	Collapsed type		1945	1946	1947	Corrected mean of three yrs.
Normal type...	107	97	0	3
<i>m₈₅</i>	103	93	5	2	21	35.1	34.6	34.9
<i>m₈₆</i>	115	97	0	3	30	34.9	18.5	22.2	25.2
<i>m₈₇</i>	113	96	1	3	24	34.7	38.8	36.7
<i>m₈₈</i>	119	75	23	2	19	23.8	..	27.6	25.7
<i>m₈₉</i>	122	88	4	8	29	31.4	43.1	37.3
<i>m₉₀</i>	123	89	5	6	19	28.6	23.2	36.5	29.4
<i>m₉₁</i>	106	92	7	1	18	30.4	41.3	...	35.9
<i>m₉₁₂</i>	108	96	3	1	6	23.8	23.8
<i>m₉₁₈</i>	112	87	1	12	6	34.7	34.7

Ovule fertility was measured in two ways: by examining embryo sacs in sectioned ovules and by measuring the number of seeds in fruits formed after pollination with pollen from normal-type plants. Each technique suffers shortcomings, yet certain conclusions are justified by a joint analysis of the two approaches.

Ovaries of open flowers were prepared for examination, and the preparations were scanned for counts of functional and nonfunctional ovules according to techniques previously described (Rick, 1946). One hundred or more ovules were counted in small samples of 8 to 10 ovaries from each mutant. These counts are presented in table 5. Here "normal" type designates an ovule whose embryo sac is fully developed and has normal appearance, "substitution" and "collapsed" types representing nonfunctional ovules. As defined, a substitution ovule is one in which a megaspore mother-cell is never differentiated, or at least never proceeds through meiosis, but is replaced in later development by a mass of undifferentiated cells of the nucellus that substitute for the embryo sac. The collapsed type refers to ovules in which maturation proceeds at least to the megaspore stage, but the female gametophyte degenerates at this or some later stage of differentiation. A low level—3 to 5 per cent—of the collapsed type is characteristic of the normal-type plants, the substitution type appearing rarely in San Marzano.

On the other hand, in all mutants except ms_6 , the substitution type appears regularly. The normal level of sterile ovules is exceeded in all save ms_6 , ms_7 , and ms_{12} , although the excess is small and is statistically significant only in ms_8 . According to this information a low level of ovule abortion might be characteristic of most of the mutants, but examination of much larger samples would be needed to reveal it.

It is noteworthy that, in keeping with the presence of substitution-type ovules in most mutants, the excess of sterile ovules in ms_8 is comprised entirely of this type. Ovules therefore degenerate at a comparatively earlier stage of development than the pollen mother-cells in this mutant. The preceding study (Rick, 1946) indicated that an unfavorable internal or external environment might induce development of the substitution-type ovule. A general suppression of growth of flower parts as observed in ms_8 might betray an adverse internal environment which might lead to this effect. Furthermore, 3 per cent or more of the substitution-type ovules are observed in all mutants whose petal lengths are significantly reduced.

The substitution and collapsed-type ovules in this survey are no doubt non-functional; but the ability of all ovules of normal appearance to function might be questioned. Nevertheless, most of them are able to develop into seeds according to the other measure of ovule fertility subsequently outlined.

Table 5 also presents the mean seed numbers per fruit obtained from matings of male-sterile and normal flowers in the backcrosses or allele test crosses previously described. Not only the variates, but also the means of the variates fluctuate markedly from mutant to mutant and from year to year. This high variability can be attributed partly to seasonal differences, to variation in the effectiveness of hand-pollination, and probably also to other factors. Because figures are not available for each mutant in each year, and because levels are much higher in 1946 and 1947 than in 1945, means for each mutant for the three-year period can be compared only if the individual means are corrected for yearly differences.

No comparable means for the normal-type plants are available. Seeds in fruits produced by self-pollination are not comparable because naturally self-pollinated flowers are not subject to a deficiency of pollen applied or pollinations made at ineffective times—factors that often reduce the seed set in hand-pollinations. Carefully emasculated and hand-pollinated flowers of the normal type might provide more comparable data; but even these are apt to provide unsatisfactory comparisons, especially in the later growth of the plant, when they often abscise or develop weakly in favor of undisturbed flowers.

Another possible disadvantage of these data is the fact that seed number per fruit is known to differ in different strains of San Marzano. The variation seen in the male-sterile mutants might be of the same nature, since they do not all necessarily represent the same strain. That the seed numbers may not always reflect differences in impairment of ovule fertility is suggested by the fact that, with one exception, they mostly vary independently of the counts of sterile ovules. The exceptional mutant, ms_8 , has, in agreement with its significantly low level of functional ovules, a low, but not the lowest, mean seed number.

Although these observations would not distinguish ovule sterilities of low grade—say 5 to 10 per cent—they do prove that an appreciably large ovule sterility exists only in the mutant *ms₈*.

RATES OF NATURAL CROSS-POLLINATION

The cultures grown at Davis in 1946 and 1947 gave an opportunity to compare the rates of natural cross-pollination of seven of the nine mutants and also to compare the rates in the two years. The plants in segregating families originally spaced 12 inches apart were thinned, after identification, to leave alternate fertile and sterile plants standing 4 to 5 feet apart in rows spaced 6 feet apart. Families of the different male-sterile mutants were scattered over an area in which fertile and male-sterile plants were represented in approximately equal numbers.

The proportion of flowers that set fruit in four plants of each mutant in each year was estimated in the following manner. Inflorescences were examined throughout the length of four branches, which were taken at random, and which originated at the crown of the plant. At the end of the growing season, when this observation is made, it is possible at Davis to distinguish accurately flowers that set fruit from those that failed to do so. The former are recognized by a greatly swollen pedicel to which the fruit may or may not be attached, and the latter, by a very slender pedicel. Two estimates of fruitfulness could thereby be obtained for each plant: the total number of fruits and the proportion of flowers that set fruit. Because parthenocarp rarely occurs in San Marzano, fruit setting in this variety is a reliable indication of pollination and consequent seed formation.

The total number of fruits set on four representative branches is affected, of course, by the total growth of the plant as well as by the rate of natural cross-pollination. The proportion of flowers that set fruit might seem, on first consideration, to be a more reliable estimate of fruitfulness because plants differed in size and, consequently, in the total number of flowers produced. This argument is valid if any differences in total flower number indicate differences in the numbers of flowers that open during the midseason period when pollen vectors are most active (see line *B*, fig. 4). But growth is usually rather uniform during this period; differences in total flower production largely reflect differences in rate of growth at the end of the season, when the amount of available water, effects of virus disease, and other factors are more apt to vary from one plant to another and from one location in the field to another. Thus, the proportion of flowers that set fruit might be affected to a great extent by the end-season growth rate of the plant as well as by the relative activity of pollen vectors among the different mutants. It would be ideal to measure the desired proportion only for the period of vector activity, but no entirely objective way of segregating the data for this period could be found. At any rate, statistical analysis of each of the two measures gives very similar indications.

The mean values obtained for the proportion of flowers that set fruit are compared graphically in figure 2. Populations of the mutants *ms₂* and *ms₃* were not available in 1946, having been discovered too late for their backcross or *F₂* families to have been included in the plantings of that year. Data for

normal-type plants are not included in figure 2; the horizontal scale of this graph is too limited to include the 30 to 60 per cent level of fruitfulness observed in the normal-type plants.

The mean values for the proportion of flowers that set fruit and for total fruits per four branches were compared by means of analysis of variance. Before so treating the figures, it was necessary to apply the square-root transformation to the values for total fruits because their number is relatively low, and the arc-sine transformation to the data for proportion of flowers that set fruit (Snedecor, 1946). These analyses are summarized in table 6.

TABLE 6
ANALYSIS OF VARIANCE OF MEAN FRUIT NUMBERS AND PERCENTAGE
OF FLOWERS SETTING FRUIT IN SEVEN MALE-STERILE
MUTANTS, 1945 AND 1946

(Mean number of fruits per four branches; percentage
of fruit by natural cross-pollination)

	Degrees of freedom	Mean number of fruits per four branches ($\sqrt{\text{---}}$ transformation)		Percentage of flowers that set fruit (angle transformation)		<i>F</i> required at 1% level
		Mean square	<i>F</i>	Mean square	<i>F</i>	
Mutants.....	6	1.491	12.12	26.38	21.45	8.47
Years.....	1	0.045	0.37	0.59	0.48	5,859
Error.....	6	0.123	—	1.23	—	—

There was close agreement in the levels of cross-pollination for the two years (fig. 2, and *F* values for years in table 6). The mutants, on the other hand, contribute a very great share of the observed variation, the *F* values being highly significant.

The natural fruit set of *ms*₅ is significantly much higher than that of any other mutant. This result might have been anticipated because fruits can be set on this mutant by self-pollination as well as by natural cross-pollination. The relative share contributed by these two sources cannot be estimated by any available data, but might be ascertained by observing the number of fertile and male-sterile offspring in the progeny derived from open-pollination of plants of this mutant surrounded by homozygous normal plants.

The lowest values were observed in the mutant *ms*₁₀. The percentage of flowers that set fruit in this mutant was significantly lower than the next lowest mutant at the 5 per cent level and lower than the remaining five mutants at the 1 per cent level. The number of fruits set by this mutant is significantly lower than that of five other mutants at the 5 per cent level and of three others at the 1 per cent level.

In the group having intermediate rates—that is, *ms*₆, *ms*₈, *ms*₉, and *ms*₁₁—differences significant at the 5 per cent level exist only between the extremes. These statistics cannot therefore decide with great certainty whether this intermediate group represents one or more infinite populations in respect to levels of natural cross-pollination; but the following comparisons of these data with measurements of anthers suggest that some of these differences may be inherent.

The very low level of ms_{10} cannot be attributed to any defect of the pistil, because transfer of pollen from the normal type to stigmas of this mutant is followed by normal fruit formation with a full complement of seed, and microscopic examination of ovaries does not reveal any appreciable abortion

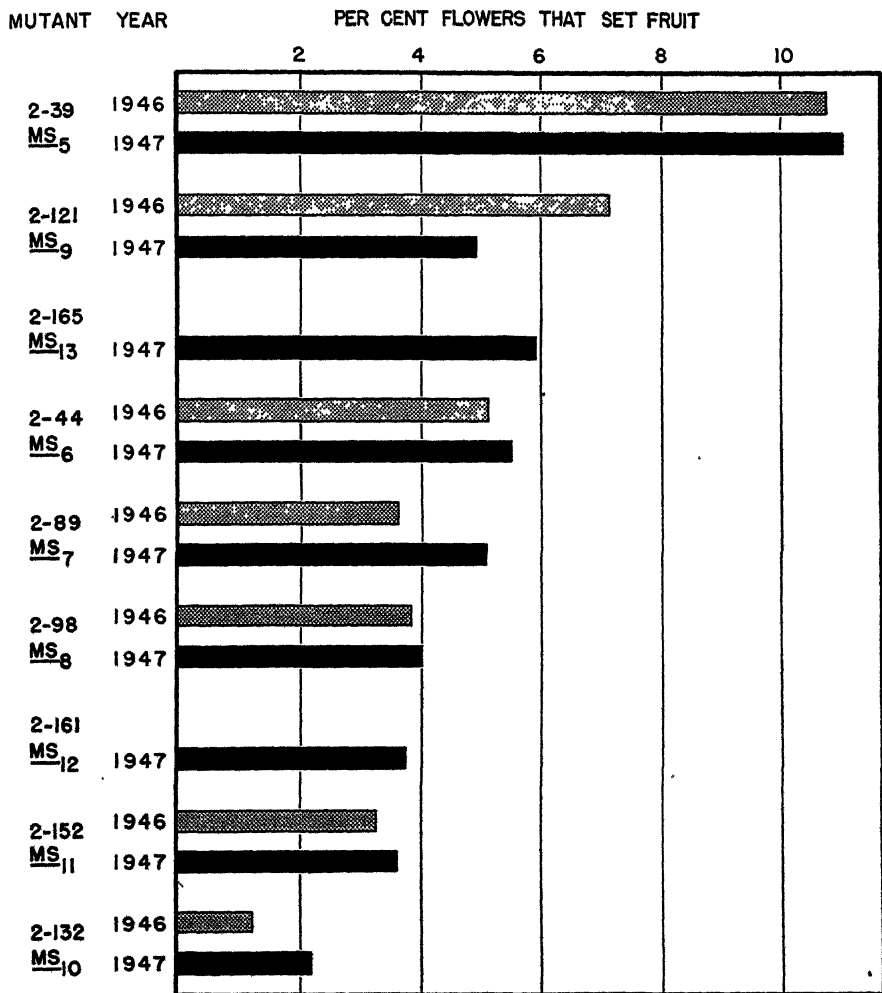


Fig. 2. Rates of natural cross-pollination of the nine mutants during 1946 and 1947.

of embryo sacs (table 5); in fact, none of the ovule sterilities observed in these mutants are high enough to affect fruit setting. It is interesting that the greatest depression of anthers occurs in this mutant, aside from the most extremely reduced flowers of ms_5 . Measurements (product of length and diameter) of anthers were compared with rates of cross-pollination to see if this relation might prevail in other mutants. Sufficient data exist to permit adding ms_{12} and ms_{13} to this group. Since ms_5 is subject to self-pollination under field conditions, it is not included in these comparisons. Means of five

flowers of each mutant were compared with the proportion of flowers that set fruit for the eight mutants, ms_6 to ms_{13} . The array strongly suggests a curvilinear relation between these two measures. If the proportion of flowers that set fruit is plotted against the log of the product of the anther-tube measurements (fig. 3), the suggestion of a curved relation is diminished, and the correlation coefficient is increased to 0.75, which, according to Fisher's (1944) t test of the correlation coefficient, is significant at the 5, but not at the 2, per cent level. The coefficient is not highly significant, and the low

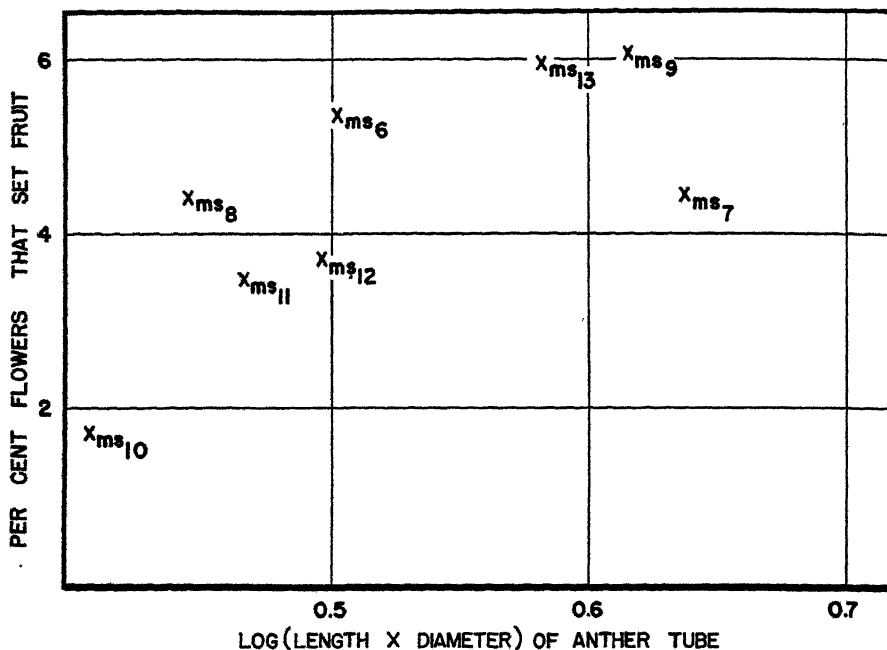


Fig. 3. Scatter diagram of eight male-sterile mutants (omitting ms_5), showing the relations between the rate of natural cross-pollination and the size of anther tube.

values of both measurements for ms_{10} doubtless contribute greatly to the high value of the coefficient; hence the limited data available strongly suggest, but do not prove beyond doubt, that the rate of natural cross-pollination decreases as anthers decrease from normal size. A study of more mutants would be needed to prove the existence of this correlation and, if it were demonstrated, to establish the nature of the relation between the two variables.

Comparisons between natural cross-pollination and such other variables of the mutant flowers as size of corolla and color of anthers do not reveal any such pronounced relation.

While the ovule fertilities give no clues, the habits of the pollen vectors provide a likely explanation of this correlation. The careful choice that honeybees make in selecting flowers to visit is an everyday observation. Similarly, the species of native solitary bees that are responsible for the transfer of tomato pollen at Davis have been observed to hover close to the tomato inflorescences and to visit only certain flowers, as if they preferred flowers of

a characteristic form or location. They might conceivably prefer tomato flowers with anthers that more nearly approximate the normal type and tend to pass by those with more deformed anthers. Accordingly, the rate of natural cross-pollination might be expected to decrease as the form and size of anthers depart from the normal type.

This hint as to the habits of the insect pollen vectors might be significant in respect to the evolutionary role of differences in flower form within species. Cross-pollination serves to maintain a high level of variability and consequent versatility of the species and the capacity to succeed in changing environments. Examples of the many devices in higher plants that ensure cross-pollination are familiar to every biologist. The present example suggests that, even in a species that is regarded as highly self-pollinating, departures from normal flower form may discourage visits of insect vectors and consequently have a lower selective advantage in a changing environment.

As in other aspects of the present investigation, the mutant ms_5 is exceptional. Even if its rate of natural cross-pollination could be estimated, comparisons of this rate with those of the other mutants would scarcely be valid because ms_5 is subject to exceedingly great variability in the reduction of its anthers. The range of variation extends from anthers that closely approach the normal type to others that are more depressed and deformed than those of any other mutant (plate 1). In contrast, the anthers of each of the other mutants show a surprising degree of uniformity in size and form.

A further hint as to vector habits is contributed by additional considerations of this exceptional mutant. In 1946 plants of this type were grown in complete isolation, being located at least 1,000 yards from any normal, fruiting tomatoes, with the hope that the seasonal setting behavior might give a clue as to the environmental effect on the expression of this gene. Preliminary observations had not, however, given any indication that the degree of anther development of ms_5 varies according to season; as a matter of fact, the full range of variation is seen on a single plant at the same season, whether summer or fall in the field, or winter or spring in the greenhouse. The seasonal fruit setting of ms_5 in isolation is illustrated by line *C* in figure 4. This line is compared in the same graph with the seasonal setting of the same mutant growing among normal-type plants (line *A*) and the mean seasonal setting of other male-sterile mutants growing among normal-type plants (line *B*). In other words, line *B* represents the seasonal variation in the amount of natural cross-pollination obtained in male-sterile mutants that do not produce any viable pollen; line *C* represents the seasonal variation in the amount of fruit setting that is conditioned by the pollen production of ms_5 alone; while line *A* represents the seasonal variation in ms_5 when fruits can develop both from cross-pollination with surrounding plants and from self-pollination. The shape of the *B* curve is quite typical of those normally obtained for seasonal fruit setting on male-sterile plants at Davis. The curve rapidly climbs to a maximum in late July, falling afterward, but at a less rapid rate to a zero value usually reached in mid-September. The *A* and *C* curves for ms_5 show generally the same shape; in fact the coincidence of maximas for all three curves is striking. The *A* and *C* curves differ from the *B* curve, however, in maintaining a continued although low level of fruit setting from

mid-September until the end of the season. This can be accepted as the only reliable estimate of natural self-pollination of ms_5 .

Since the anthers of ms_5 vary greatly in form and ability to produce functional pollen, it might be presumed that the seasonal pattern of fruiting of this mutant (curve *C*) could be explained by a variable proportion of pollen-producing, self-pollinating flowers corresponding to the levels of fruit set at various parts of the season. But no such variation has been observed: instead, the same general proportion of flowers with different degrees of anther deformity appears at all times of the year. The great similarity in shape of the three curves suggests a more likely explanation. Curve *B* must represent the

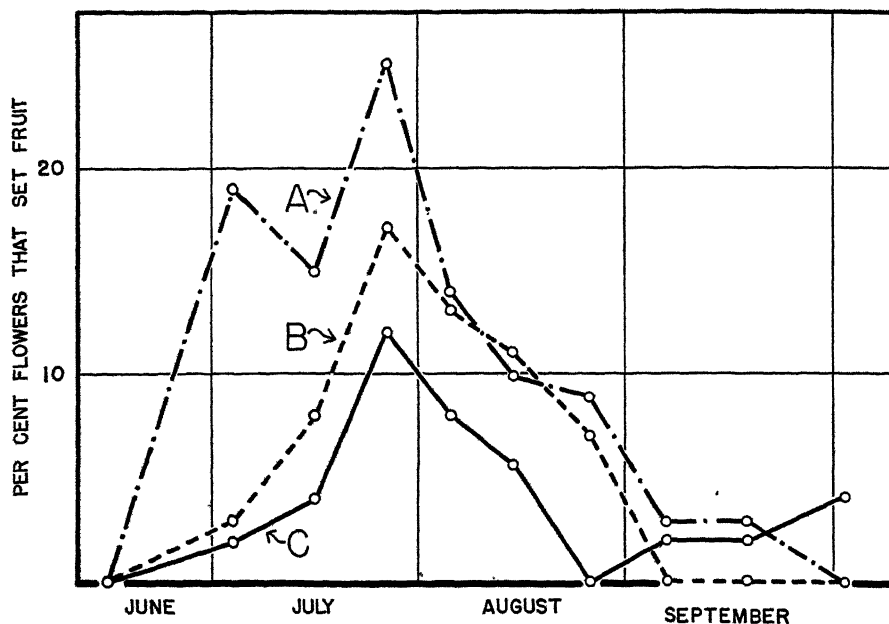


Fig. 4. Seasonal variation in the rate of natural cross-pollination: *A*, ms_5 surrounded by fertile plants; *B*, mean values for plants of other male-sterile mutants surrounded by fertile plants; *C*, ms_5 isolated from fertile plants.

seasonal pattern of activity of the tomato pollen vectors because the mutants represented in this curve will set fruit only if functional pollen is transferred to them. Also, the increased levels of curve *A* in respect to curve *C* must be explained in terms of insect vector activity. It therefore seems more in keeping with the evidence that *insect vectors are mainly responsible for the pollination and the resulting fruit setting of the ms_5 mutant, even if it is isolated by distance from fertile tomatoes.*

Another fact that lends support to this interpretation is the observation that a considerable number of fruits was set on plants of ms_5 growing close to the plants of ms_5 in the isolated plot. The mutant ms_5 is known to be completely male-sterile because it has never set fruits containing seeds when isolated from all sources of viable tomato pollen. The fruits set on ms_5 in this planting must therefore have resulted from the transfer by insect vectors of pollen, limited though it must have been, from ms_5 .

If these observations have been interpreted correctly, they reveal a keen ability of the native solitary bees to select the pollen-bearing flowers of the ms_s mutant. If the insect is guided by the shape and size of the anthers, as the earlier analysis suggests, the results might not seem so extraordinary because, in tending to visit the flowers that more nearly resemble the normal type, the insect would have a greater chance of finding one that would yield normal pollen, and would thereby increase the fruit setting of this mutant.

The foregoing considerations of the fruit setting of the male-sterile mutants reveal surprising suggestions concerning the habits of the pollen vectors. Also unexpected was the information regarding the amount of pollen transferred on a single visit and the distance over which a vector might forage on a single flight gained in an analysis of the pollinating relations of the dl mutant (Rick, 1947). Additional information—for instance, the relative activity of vectors in different localities—can be gained by appropriate experiments with these mutants.

FREQUENCY OF MALE-STERILE MUTATIONS

The present survey adds nine ms genes to four that had already been discovered in *Lycopersicon esculentum*. In 16 male-sterile plants collected in tomato fields, therefore, 13 different genes are involved. The data are still too meager to provide an estimate of the total number of genes for male sterility in this species, but the number is probably very much higher than 13.

In addition to these mutants, another one, probably representing a new gene, has been discovered recently in the variety Earliana. Preliminary genetic studies indicate that this male sterility is probably likewise conditioned by a single recessive gene.

The abundance of genes for male sterility in tomatoes is by no means unique in flowering plants. Such genes have been reported in at least 19 different species in widely separated taxonomic groups. Furthermore, in species which have been subjected to more intensive genetic study, they have been recovered with comparable frequency—for instance, at least 23 genes for male sterility are known in maize (Beadle, 1932; Emerson, Beadle, and Fraser, 1935).

On first thought the repeated recovery of ms_s might be attributed to its ability to produce a small quantity of functional pollen. While this exceptional ability increases the reproductive capacity of this mutant, it does so only to a slight extent. Though the amount of seed produced by self-pollination of ms_s has never been measured, it cannot amount to more than 5 per cent of that produced by fertile plants. Now, if it is assumed that Ms_sms_s plants reproduce at the same rate as the other fertile plants in the field, the proportion of such plants should be halved in each generation. One quarter of their offspring will be ms_sms_s , which will breed true, but reproduce at 5 per cent or less of the rate of Ms_sms_s and other fertile plants. This additional yield of homozygotes accumulated from self-pollination of homozygotes amounts to only about 10 per cent of the number that are yielded by the heterozygotes. Furthermore, in collecting seed, seedsmen may tend to avoid plants that are as unfruitful as ms_sms_s .

It seems more likely, therefore, that this gene was recovered repeatedly because a large proportion of the heterozygote was included, as a result of chance

or higher mutation rate, in the plantings that were the predecessors of the ones in which unfruitful plants were sought.

Absence of Cytoplasmically Male-sterile Mutants. No cytoplasmically male-sterile mutants were discovered in the samples of unfruitful tomato plants. Since the tomato is very highly self-pollinated, all factors that cause sterility, whether of pollen or ovule or both, would enjoy equal opportunity of discovery.

Cytoplasmic male sterility is generally much less common in cultivated plants than genic male sterility, but examples are known in maize (Rhoades, 1933), onions (Jones and Clarke, 1943), and sugar beets (Owen, 1945). If the cytoplasm regulates expression of the known male-sterile mutants in tomatoes, it is favorable toward all, and, in this sense, all investigated lines might be said to have the appropriate cytoplasmic factor.

Male sterility determined exclusively by the cytoplasm (as in maize) could be reproduced at a rate that would depend on the proportion of natural cross-pollination. In tomatoes, because the rate of natural cross-pollination is very low, the reproductive rate of this type would be correspondingly low. If the determination is based on an interaction of a cytoplasmic factor and one or more genes (as in onions and sugar beets), reproduction at a much higher rate could occur by self-pollination of the fertile heterozygotes having the sterile-type cytoplasm. But any masking of the male-sterile genes by the non-sterile cytoplasm would serve to prevent recovery of the male-sterile phenotypes; the genes of such plants would never attain phenotypic expression except for the small extent to which they might be transferred by natural cross-pollination to plants having the appropriate sterile cytoplasm. In highly self-pollinated crops, therefore, male sterility determined by cytoplasmic factors would tend to disappear more quickly than that determined solely by genes; and it is therefore not surprising that no cytoplasmic factors were found in this crop.

Absence of Female-sterile Mutants. The absence of female sterility in tomatoes is matched by a similar situation in other genetically analyzed cultivated plants. The term female sterility here refers to an effect on ovule production analogous to that on pollen production in male-sterile mutants; in other words, it is a complete breakdown in megasporogenesis or development of the female gametophyte without adverse effect on the development of functional pollen. Emerson, Beadle, and Fraser (1935) list over 300 genes, including 23 for male sterility, known in maize, but only one (silkless) is female-sterile. Several other mutants, such as barren stalk, are effectively female-sterile, but in them, the entire pistillate inflorescence is absent, and they are therefore not strictly comparable to the male-sterile mutants, in which the sex organ is present but fails to produce functional gametes.

Although female-sterile mutants have not been found in tomatoes, others are known that are both female and male-sterile (Rick, 1945a). Because the latter have never appeared in segregating families, it has been impossible to obtain any information concerning their heredity. It seems quite likely, however, as with the mutants which can be bred, that they are genetically determined.

It is known that mature pollen and the developmental stages leading to the

production of pollen are more sensitive to unfavorable environments than those of embryo sacs. Wheat, for instance, can be emasculated by chilling the emerging heads (Suneson, 1937); the pollen of *Sorghum* can be destroyed without injury to other floral parts by heat treatments (Stephens and Quinby, 1933); and heat treatments applied to maize as early as the seedling stage will effectively induce male sterility (Jones, 1947). Now, if the effects of the genes on gamete formation and also those on the size of floral parts accrue as pleiotropic effects of some earlier and more fundamental action, the absence of true female sterility might be understood. In the light of these physiological experiments, the less severe action might lead to male sterility only, and the more drastic action to total sterility, an effect solely on the female gametes being impossible in this scheme.

USE OF THE MALE-STERILE MUTANTS FOR CONTROLLED CROSS-POLLINATION

Any of the mutants described in this study could be used advantageously as pistillate parents in controlled cross-pollination. With one exception all are completely pollen-sterile; again, with one exception, they show no great degree of ovule sterility. Some of them, however, present certain advantages that are not shared by others.

In producing large numbers of male-sterile segregates of any mutant, ease of identification would be an important consideration. To be sure, all the mutants can be identified macroscopically by prodding the anthers with a blunt instrument, like the tips of a forceps, in order to detect whether large masses of normal pollen are formed. But such a technique is much more tedious than identification of the segregates by means of discernible differences in color, size, or shape of anthers.

Exsertion of the stigma is another important practical consideration, for it permits much easier hand-pollination than nonexserted stigmas which are sunken below the apex of the anther tube. In respect to these two factors—ease of macroscopic identification and degree of stigma exsertion—mutants ms_5 , ms_8 , ms_{10} , and ms_{12} would be most desirable; ms_9 and ms_{13} would be least useful, while ms_6 , ms_7 , and ms_{11} would occupy an intermediate level of desirability for the production of hybrid seed.

A third factor that deserves consideration is the rate of natural cross-pollination to which the mutant is subject. Though the amount of seed that can be produced in this way is limited in many localities, it might provide a sufficiently welcome addition to the hybrid seed produced by hand-pollination to merit the interplanting of the male-sterile mutant and the fertile pollen parent. In respect to this factor, the lesser the degree of anther deformity, apparently the greater the rate of cross-pollination and consequently the more desirable the mutant. This consideration might disqualify ms_{10} because of its much lower rate. It might be well, therefore, to select mutants such as ms_6 and ms_7 , which satisfy the first two qualifications, and, at the same time, are subject to a relatively high rate of insect pollination.

At this point ms_9 again deserves special consideration. Because it can produce functional pollen, it offers the unique advantage of being self-pollinating and therefore reproducible in 100 per cent of the progenies derived from self-

pollination. In contrast, the maximum yield of male-sterile individuals of all other mutants is the 50 per cent obtainable from backcrosses. From the standpoint of large-scale production of F_1 hybrid seed, the advantage of this mutant does not lie so much in the higher yield of sterile plants as it does in the elimination of any need for growing segregating populations to the flowering stage for identification of the sterile segregates before they are planted in the field. Exclusively male-sterile progenies of *ms*, can be produced by controlled self-pollinations or by open pollinations of the sterile plants grown under complete isolation, or by a combination of both methods. Extraction of pollen is tedious and might seriously limit the feasibility of self-pollination by hand. On the other hand, the mechanical pollen collector invented by Cottrell-Dormer (1945) might possibly be used to advantage here.

By the very token of pollen production, which permits the generation of 100 per cent male-sterile populations, the use of *ms*, as a pistillate parent in the production of hybrid seed would be at a disadvantage because such hybrid seed would be contaminated by a low proportion of seed resulting from self-pollination. Though very intensive cross-pollination might serve to reduce this self-contamination to a low level, even a very small proportion of such contaminants would be objectionable because they would necessarily be male-sterile and unfruitful. This difficulty might be circumvented in several ways. First, the proportion of contamination might be reduced or completely eliminated by pollinating only the flowers that have the greatest reduction of anthers and by pulling off the remaining ones that approach the normal type. As previously described, the degree of abnormality of anthers in this mutant is inversely related to the amount of functional pollen produced. Second, the undesirable sterile contaminants could be identified by the use of a recessive seedling character, as previously suggested for a similar purpose by Currence (1944). If, for example, the male-sterile stock were homozygous for a_1 , the gene determining the absence of anthocyanin pigment, and the desired pollen parent were true-breeding for the dominant allele, the F_1 hybrid seedlings could be distinguished by the purplish color of their hypocotyls from the self-contaminants, which would have green hypocotyls.

Another development that greatly affects the use of genetic male sterility in producing F_1 tomato hybrids is the recent description of a mechanical pollen collector by Cottrell-Dormer (1945). Heretofore considerable time has been required in the tedious operation of forcing pollen from the anthers of the desired pollen parent. This simple device, consisting of an electric vibrator, efficiently shakes the pollen from the anther tubes into replaceable glass cups. One can collect sufficient pollen with it to last for an entire day of pollinating. If this device were used to collect pollen from the staminate parent, and if male-sterile lines were used to circumvent emasculation of the pistillate parent, the cost of hybrid tomato seed production should be greatly reduced.

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